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Research article

Ameliorative effects of Monascus-fermented hawthorn extract on a high-fat diet-induced rat model of non-alcoholic fatty liver disease

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ABSTRACT

Objective: The aim of this study was to elucidate the effects of fermented hawthorn extract on high-fat diet (HFD)-induced non-alcoholic fatty liver disease (NAFLD) in rats, and explore the possible underlying mechanisms.

Methods: A total of 42 male adult Sprague-Dawley rats were randomly divided into five groups: normal control group (given a normal feed diet and distilled water by gavage), NAFLD model (given HFD and distilled water by gavage), low-, medium-, and high-dose fermented hawthorn extract treatment groups (given HFD and different doses of fermented hawthorn extract by gavage). After 12 weeks of gavage administration, changes in body weight, liver/body weight ratio, serum liver enzymes, as well as triglyceride (TG) content and oxidative stress levels in rat liver tissueswere detected. Histological evaluation was performed to observe the degree of fat accumulation (steatosis). qRT-PCR and western blotting were performed to detect the mRNA and protein expression of cytochrome P4502E1 (CYP2E1, a key enzyme associated with lipid peroxidation), and lipogenic factors (sterol regulatory element-binding protein 1c (SREBP-1c) and fatty acid synthase (FAS)) in rat liver tissues.

Results: Fermented hawthorn extract significantly reduced the body weight, decreased the levels of liver enzymes, improved hepatic steatosis, and exhibited obvious antioxidant effects. Fermented hawthorn extract also significantly down-regulated the mRNA and protein expression levels of CYP2E1, SREBP-1c and FAS.

Conclusion: Our findings suggested that fermented hawthorn extract can markedly reduce body weight, ameliorate HFD-induced NAFLD in rats, and exhibits significant antioxidant effects. Its underlying mechanism may depend on the inhibition of CYP2E1, SREBP-1c, and FAS expression.

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1. Introduction

The global prevalence of non-alcoholic fatty liver disease (NAFLD) is approximately 25 %, which poses a serious threat to social development and human health, and has become a major global health problem. In the widely accepted "multiple hits" theory of pathogenesis, the initial ''hit'' can be explained by impaired lipid metabolism, where liver parenchymal cells accumulate excessive triacylglycerol in lipid droplets, subsequently causing hepatic steatosis. The second "hit'' is oxidative stress. Despite some agents have been proved to be effective, such as some antidiabetic drugs, stearoyl-CoA desaturase 1 inhibitors, fatty acid synthase inhibitors over the past 10 years, AMP-activated protein kinase activators and thyroid hormone receptor-β agonists, there are still no approved therapies to treat NAFLD. At present, the mainstay of treatment for NAFLD is lifestyle modification using diet and exercise, with the ultimate goal of weight loss [1]. Sustained weight loss is challenging because it requires a transformation of ingrained behaviour patterns. The efficacy of traditional Chinese medicine in treating chronic diseases such as NAFLD is stable, and has minimal side effects; Moreover, traditional Chinese medicine contains multiple active ingredients that can exert pharmacological effects through multiple pathways and targets [2].

Hawthorn has been used for medicinal and edible purposes for at least 2000 years. Previous experimental study of our group showed that hawthorn can ameliorate fatty liver disease, hyperlipidemia, abnormal liver enzymes, and oxidative stress in rats [3,4]. A clinical study suggested that hawthorn can reduce body weight, improve insulin resistance, and NAFLD [5]. Fermentation can improve the taste of hawthorn, substantially increase the production of active substances, and enhance its biological activity [6]. In a previous study carried out in China, researchers performed *Monascus* fermentation of hawthorn to increase the production of active substances [7]. According to the *Monascus* fermentation process conditions [7], the present study aimed to observe the pharmacological effects of *Monascus*-fermented hawthorn extract on NAFLD in rats, and explore the underlying mechanisms, so as to provide a basis for its clinical application.

2. Materials and methods

2.1. Animals

A total of 42 specific pathogen free-grade male Sprague-Dawley rats (weighing 160–220g) were provided by Guangdong Medical Laboratory Animal Center (animal license number SCXK (Guangdong) 2018 -0002). All rats were routinely housed at a constant room temperature of 21 ± 1 °C and relative humidity of 55 ± 5 % with a 12 h dark/light cycle. They were free access to water, and acclimatized for 1 week. All animal procures were were approved by the Animal Ethics Committee of Huazhong University of Science and Technology Union Shenzhen Hospital (No.ky-2018-010302).

2.2. Reagents and instruments

Alanine aminotransferase (ALT) assay kit (Shanghai Fosun Long March Medical Science Co., Ltd., Shanghai, China); aspartate aminotransferase (AST) assay kit (Shanghai Fosun Long March Medical Science Co., Ltd., Shanghai, China); triglyceride (TG) assay kit (batch number:A110-1-1, Nanjing Jiancheng Bioengineering Institute, Nanjing, China); Trizol reagent (batch number: JR 00546, TaKaRa, Kyoto, Japan); total RNA extraction kit (batch number: 03505, Tiangen Biotech (Beijing) Co., Ltd., Beijing, Tianjin); reverse transcription kit (batch number: 00238763, Thermo Fisher Scientific, USA); SYBR Green PCR Master Mix kit (batch number: 441900, Toyobo Company, Japan); hematoxylin-eosin (HE) staining kit (batch number: 2017042501, Leagene Biotechnology, Beijing, China); Oil Red-O stain kit (Solarbio, Beijing, China). XSP-C204 optical microscope (Wuhan Optoelectronics Instrument Co., Ltd., Wuhan, China); large-scale automatic biochemical immunoassay analyzer (CI 16200, Abbott, Chicago, IL, USA); ZX6006 desktop high-speed centrifuge (Jouan, France); TS-1 horizontal shaker (Beijing Jinshisu Apparatus Manufacturing Co., Ltd., Beijing, China); 164–5050 electrophoresis apparatus (Bio-Rad, USA); ABI7500 Real-time polymerase chain reaction (PCR) system (Thermo Fisher Scientific, USA); CM 1905frozen slicer (Leica, Wetzlar, Germany).

2.3. Preparation of fermented hawthorn extract

The hawthorn (net hawthorn, Sinopharm Group Co., Ltd., Shanghai, China, place of origin: Shandong) was taken, washed, and soaked in water for 30 min, heated, and boiled for 30 min. Then the mixture was collected into a flask and pasteurized. Subsequently, the flask was inoculated with 10 % of *Monascus purpureus* (M212, Shanghai Institute of Industrial Microbiology Shanghai, China), and placed in a thermostatic shaker at 28 °C for liquid culture fermentation. After 10 days of fermentation, the residues were removed by filtrating. After 15 days of fermentation, fermented hawthorn extract was obtained, and diluted with distilled water to different doses.

2.4. Animal grouping and model establishment

After acclimatization for 1 week, rats were randomly divided into 5 groups: normal control group (n = 9), NAFLD model group (n = 9), and low-dose (n = 8), medium-dose (n = 8), and high-dose fermented hawthorn extract treatment groups (n = 8). The rats in the normal control group were fed with standard feed. Rats in the remaining 4 groups were given the high-fat diet (HFD) containing 10 % lard, and 2 % cholesterol [8]. Rats in the normal control and model groups were administrated with distilled water (10 ml/kg/d) by gavage. Rats in the low-, medium-, and high-fermented hawthorn extract groups were administrated with different doses of fermented

hawthorn extract by gavage. The dose of fermented hawthorn extract used in this experiment was calculated based on the dose used in human adults. A human dose of 30g/60 kg body weight was calculated to be equivalent to a rat dose of 317 mg/100g body weight (medium dose). The low dose was half of the human-equivalent dose, while the high dose was double the human-equivalent dose, i.e. original medicinal materials contained in low, medium and high doses of fermented hawthorn extract were 158.5mg/m1, 317 mg/ml and 634 mg/ml, respectively. The doses of fermented hawthorn extract administered to the animals were determined according to our previous study [4].

2.5. Sample collection and serum biochemical detection

After 12 weeks of gavage administration, rats were fasted for 12 h but allowed free access to water. The body weight of each rat was measured. Then, the rats were sacrificed using intraperitoneal injection of 3 % sodium pentobarbital (30 mg/kg). The blood was taken from the abdominal aorta, and centrifuged to obtain the serum for detecting the levels of liver enzymes (ALT and AST) with corresponding kits. The liver of each rat was removed quickly and weighed, the liver weight and the liver/body weight ratio was then calculated.

2.6. Histological evaluation

Liver tissues were taken from the liver lobe of each rat, and fixed in 10 % formalin solution, embedded and sliced into tissue sections, and stained with HE according to manufacturer's instructions. Liver tissue was embedded in optimal cutting temperature, cut into 10 μm thick sections by using a cryotome, and stained with Oil Red-O according to manufacturer's instructions. The degree of hepatic steatosis in rat liver tissues was observed under an optical microscope. The rest of the liver tissues was placed in a cryo-tube, and stored in a −80 °C refrigerator for later use. All liver biopsies were evaluated by a blinded pathologist and scored using NAFLD Activity Score (NAS) [9].

2.7. Detection of TG content and oxidative stress markers in rat liver tissues

Part of the liver was homogenized. The TG content, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) activities, as well as malondialdehyde (MDA) content were determined according to corresponding kits.

2.8. Detection of lipid metabolism-related genes in the liver tissues using quantitative RT-PCR (qRT-PCR)

qRT-PCR was performed to detect the mRNA expression levels of cytochrome P4502E1 (CYP2E1), and sterol regulatory element-binding protein 1c (SREBP-1c) and fatty acid synthase (FAS) in rat liver tissues. Total RNA was extracted from liver tissues by using TRIzol reagent according to the manufacturer's instructions, which was reverse transcribed to cDNA using the reverse transcription kit. After completion of the amplificatoin reaction, melting curves of the PCR products were generated, the melting step was as follows: 95 °C for 15 min, 60 °C for 60 min, 95 °C for 15, and ramping from 60 °C to 99 °C with ramping rate 0.05 °C/s β-actin was used as the internal reference gene, the relative expression of the target gene was determined by the ratio of the initial copy numbers of the target gene and β-actin. Primer sequences are shown in Table 1.

2.9. Detection of protein expression of CYP2E1, SREBP-1c and FAS using Western blot analysis

The total protein was extracted from the liver of rats by total protein extraction kit, and the protein concentration was detected by the BCA method. 50 μg protein samples were loaded, separated by 15 % SDS-PAGE gel (10 % separating gel, 5 % stacking gel), and transferred onto polyvinylidene fluoride (PVDF) membrane. Then the PVDF membrane was blocked with 5 % BSA blocking solution for 2 h, incubated with CYP2E1, SREBP-1c and FAS antibodies at 4 °C overnight. After washing with TBST four times for 5 min each, the PVDF membrane was incubated with secondary antibodies at room temperature for 1 h. The protein blots were developed using a charge-coupled device imager. The gray value of each band was analyzed using Image J software. GAPDH was used as the internal reference protein.

Table 1
Primer sequences used for qRT-PCR.

Gene	Forward primer (5′-3′)	Reverse primer(5′-3′)
CYP2E1	TGAGACCACCAGCACAACTC	TGGATCTCATGCACCACAGC
FAS	AGGGTTTGGAGTTGAAGAGGAG	CACCTGCACTTGGTATTCTGG
SREBP-1c	CCTCACTCCCTCTGATGCTTC	GTCAGCTTGTTTGCGATGTCTC
β-Actin-F	CATGTACGTTGCTATCCAGGC	CTCCTTAATGTCACGCACGAT

CYP2E1, cytochrome P4502E1; SREBP-1c, sterol regulatory element-binding protein 1c; FAS, fatty acid synthase.

2.10. Statistical analysis

The data were analyzed using SPSS 24.0 software. All data are expressed as the mean ± SD and analyzed by one-way analysis of variance (ANOVA). A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Fermented hawthorn extract reduces body weight, liver weight, liver/body weight ratio, and elevated serum liver enzymes in rats with NAFLD

One rat in the medium-dose fermented hawthorn extract group died after gavage administration, the rest of rats survived until the termination of the study. After 12 weeks of HFD feeding, the body weight, body weight gain, liver/body weight ratio were significantly higher in the model group than in the normal control group ($p < 0.05$, $p < 0.01$). The high- and medium-dose groups exhibited a significant decrease in body weight, body weight gain, liver/body weight ratio compared to the model group ($p < 0.05$, $p < 0.01$, Table 2). The low-dose group displayed a trend towards a decrease in body weight, body weight gain, liver/body weight ratio, but no significant difference was found between the low-dose group and model group (Table 2). Rats in the high-, medium- and low-dose groups had decreased liver weights when compared to the model group ($p < 0.05$, Table 2).

Serum AST and ALT contents were significantly increased in the model group compared with the normal control group ($p < 0.01$), which were significantly decreased in the high-dose group compared with the model group ($p < 0.05$, $p < 0.01$, Fig. 1A and B). A tendency of a decrease in serum AST and ALT contents was observed in the low-, medium-dose groups, but there was no significant difference between the low-, medium-dose groups and the model group ($p < 0.05$, Fig. 1A and B).

3.2. Fermented hawthorn extract prevents hepatic steatosis in rats with NAFLD

When observed with the naked eye, liver of rats in the normal control group showed reddish brown color, while the liver color of the model group appeared yellow, and the liver was swollen. Compared with the model group, liver color changed red in varying degrees, which was redder in the high-dose group (Fig. 2).

HE staining showed that in the normal control group, the structure of the hepatic cords was normal, which was arranged radially with normal hepatocyte morphology. In the model group, the structure of the hepatic cords was disordered, lipid droplets of varying sizes and numbers were found in most of the hepatocytes, and some cells exhibited a displaced nucleus, and lobular inflammation was observed. In the high-, medium- and low-dose fermented hawthorn extract groups, liver lipid deposition and lobular inflammation were obviously improved, and the number of lipid droplets in the hepatocytes was markedly reduced compared with the model group. Additionally, the contour of some cells was normal with intact structure. The above-mentioned improvement was more pronounced in the high-dose group, i.e. the hepatic cord arrangement was neater in the high-dose group than in the low-dose group, and the contour of hepatocytes was clearer in the high-dose group than in the medium and low-dose groups (Fig. 3).

The NAS scores of each group are shown in Table 3. The NAS score of the model group was higher than that of the normal control group, which was also higher than that in the high-, medium- and low-dose groups (Table 3).

Oil red-O staining of sections of rat liver tissues showed that the liver structure of the normal control group was normal without obvious lipid deposition, whereas obvious lipid deposition and markedly increased area of Oil Red-O staining were seen in the model group ($P < 0.01$, Fig. 4A and B). Compared with the model group, the area of Oil Red-O staining was obviously decreased in the high-dose group ($p < 0.05$, Fig. 4A and B). Decreased area of Oil Red-O staining was also found in the low- and medium-dose groups.

Table 2
Effect of fermented hawthorn extract on changes in body weight and liver/body weight ratio in rats with NAFLD (mean ± SD).

Groups	Number	Body weight (g)	Weight gain at 12 weeks (g)	Liver weight (g)	liver/body weight ratio (%)
Normal control group	9	600.99 ± 66.49[a]	352.12 ± 58.60[a]	9.05 ± 1.51[b]	2.58 ± 0.22[b]
t value/P value		2.235/0.040	2.390/0.029	6.276/0.001	6.651/0.00
Model group	9	666.54 ± 57.62	418.36 ± 58.97	17.88 ± 3.94	4.28 ± 0.74
Low-dose fermented hawthorn extract group	8	625.55 ± 81.88	374.35 ± 79.49	14.90 ± 3.33	3.97 ± 0.26
t value/P value		1.205/0.247	1.307/0.211	1.589/0.112	1.173/0.282
Medium-dose fermented hawthorn extract group	7	597.11 ± 31.89[a]	332.41 ± 48.08[b]	13.02 ± 1.56[b]	3.94 ± 0.26
t value/P value		2.852/0.013	3.125/0.07	3.377/0.006	1.291/2.24
High-dose fermented hawthorn extract group	8	550.23 ± 50.69[b]	307.43 ± 44.29[b]	10.42 ± 1.22[b]	3.40 ± 0.19[b]
t value/P value		4.393/0.001	4.338/0.001	5.127/0.001	3.447/0.007

NAFLD, non-alcoholic fatty liver disease.
[a] $p < 0.05$.
[b] $p < 0.01$, vs the model group.

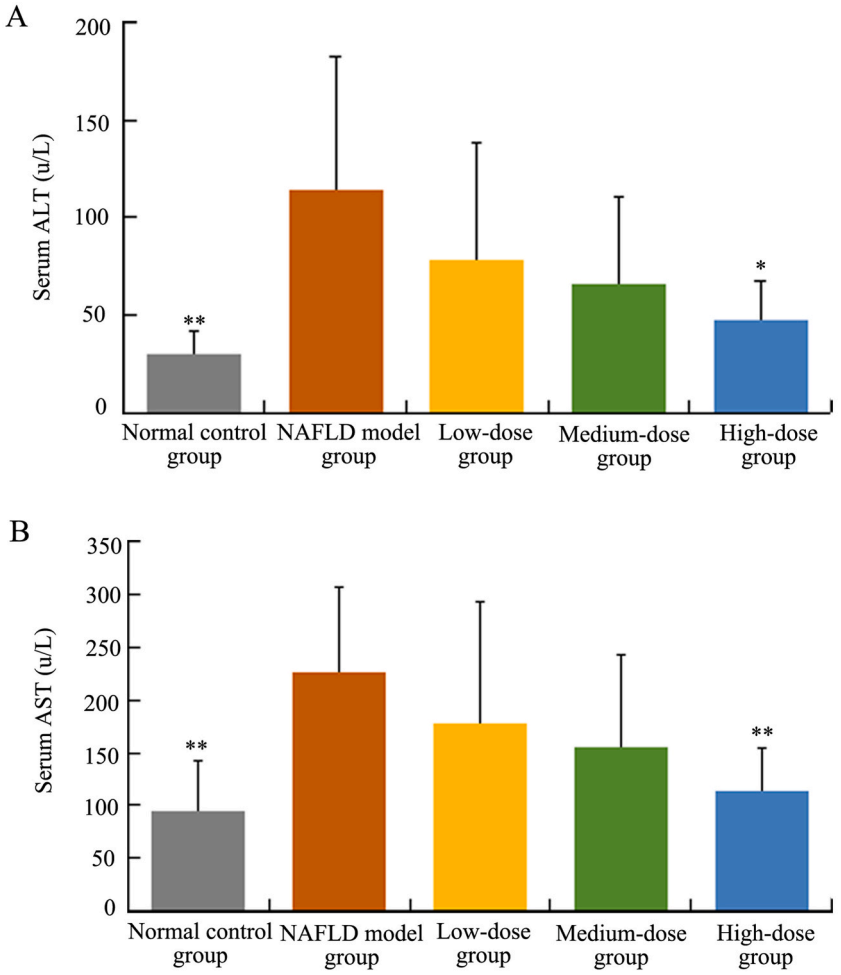

Fig. 1. Effect of fermented hawthorn extract on serum liver enzymes in rats with NAFLD. A: serum ALT levels; B: serum AST levels. *$p < 0.05$, **$p < 0.01$, vs. the model group. NAFLD, non-alcoholic fatty liver disease; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

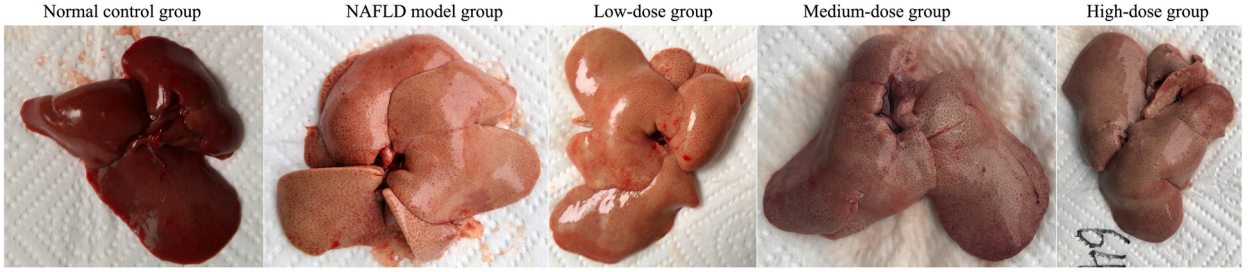

Fig. 2. Naked eye observation of rat livers in each group.

3.3. Fermented hawthorn extract reduces TG content and oxidative stress levels in liver tissues

TG content was significantly increased in rat liver tissues in the model group than in the normal control group, which was significantly decreased in the medium- and high-dose fermented hawthorn extract groups than in the model group (both $p < 0.05$, Fig. 5A).

SOD and GSH-Px activities were significantly decreased, MDA contents were significantly increased in rat liver tissues in the model group than in the normal control group ($p < 0.05$, $p < 0.01$, Fig. 5B–D). Compared with the model group, SOD and GSH-Px activities were significantly increased, and MDA contents were significantly decreased in rat liver tissues in the high-dose group ($p < 0.05$, $p <$

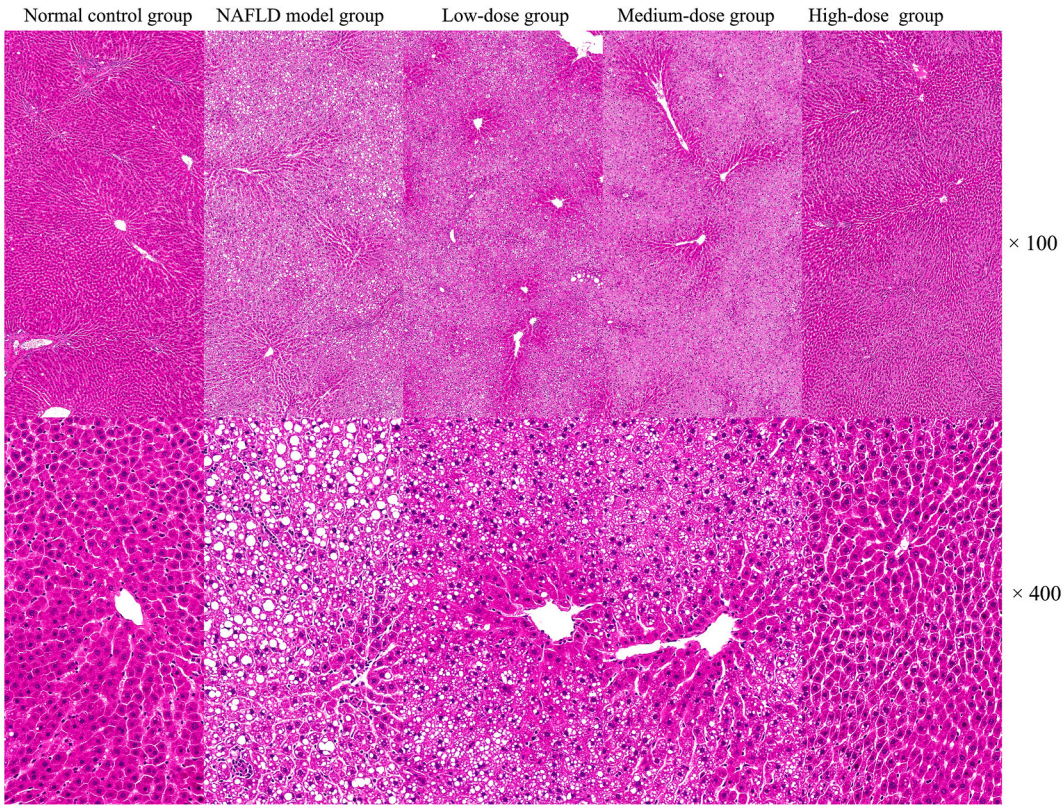

Fig. 3. Hematoxylin-eosin (HE) staining of liver sections from each group.

Table 3
NAFLD Activity Score of each group.

Group	Steatosis	Lobular inflammation	Hepatocyte ballooning
Normal control group	**0**	**0**	**0**
Model group	**3**	**2**	**0**
Low-dose fermented hawthorn extract group	**2**	**1**	**0**
Medium-dose fermented hawthorn extract group	**2**	**0**	**0**
High-dose fermented hawthorn extract group	**1**	**0**	**0**

0.01), whereas no significant difference was found between the low-, medium-dose groups and the model group (Fig. 5B–D).

3.4. High-dose fermented hawthorn extract down-regulates the mRNA and protein expression of CYP2E1, SREBP-1c and FAS in rat liver tissues

Since the effect of the low- and medium-dose fermented hawthorn extract was not obvious, so we only detected the effect of high-dose fermented hawthorn extract on the expression of CYP2E1, SREBP-1c and FAS in rat liver tissues using qRT-PCR and Western blot analysis. The results showed that compared with the normal control group, mRNA and protein expression levels of CYP2E1, SREBP-1 and FAS were significantly up-regulated in the model group(all $p < 0.05$, Fig. 6A–C,7). Treatment with high-dose fermented hawthorn extract significantly inhibited the mRNA and protein expression levels of CYP2E1, SREBP-1c and FAS in rat liver tissues (all $p < 0.05$, Fig. 6A–C,7, Supplement Fig. 1).

4. Discussion

Non-alcoholic fatty liver disease (NAFLD), the most common chronic liver disease, has become a health burden worldwide with no effective treatment [1,10]. Hawthorn is a well-known traditional Chinese medicine used to treat dyspepsia syndrome, cardiovascular disease, and hyperlipidemia, which has complex composition. Hawthorn is one of the most frequently used medicines in the treatment of NAFLD, which has significant efficacy and no significant toxic side effects within the usual dose range [11,12]. However, at present, edible hawthorn berries for weight loss have limited efficacy, the taste experience is not good, so the use of suitable fermentation

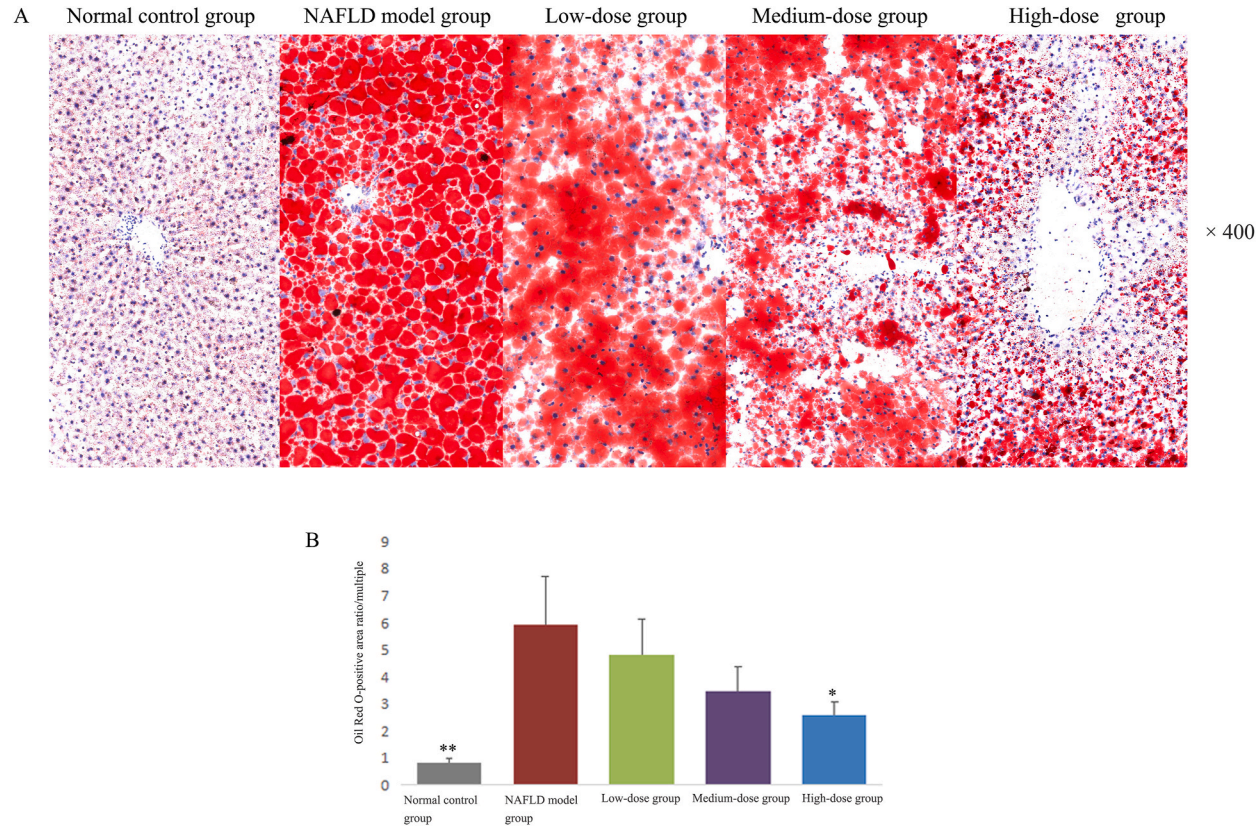

Fig. 4. Oil red-O staining of liver sections from each group. A: Histological section observation of liver tissues by Oil Red-O staining. B: Quantification of Oil Red-O positive areas. $*p < 0.05$, $**p < 0.01$, vs. the model group. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

processing methods are needed to enhance the efficacy of hawthorn and improve the taste experience. Fermentation is one of the popular methods used in fruit processing in South China, and the fermentated hawthorn has gained popularity. There are many different fermentation methods. A previous study confirmed that bioactive compounds in the hawthorn has increased several times after *Monascus* fermentation, *Monascus* fermentation can destroy the matrix of insoluble dietary fiber in the plant, expose the internal structure and reduce the crystallinity [13]. In this study, hawthorn was fermented by using *Monascus. Monascus*-fermented hawthorn extract looks like fruit pulp, smells fragrant and mellow, without bitter taste and with light sour taste and sticky texture, which is suitable for long-term consumption.

In this study, a rat model of NAFLD was successfully established, which was proved by TG accumulation in the liver. Histopathological observation also showed severe steatosis in the NAFLD rat model. Additionally, the results showed that ALT and AST levels were significantly elevated in HFD-induced rats. The above-mentioned results indicate that HFD-induced rats developed metabolic disorders, hepatic steatosis, and hepatotoxicity, which are consistent with findings from previous studies [8,14].

In this study, we found that fermented hawthorn extract significantly reduced the body weight and liver/body weight ratio in HFD-induced NAFLD rats. Histopathological observation of liver tissues showed significant improvement in hepatic steatosis, and reduction in fat deposition. Serum biochemical analysis showed that fermented hawthorn extract significantly reduced HFD-induced serum transaminase elevations, suggesting that fermented hawthorn extract treatment can ameliorate HFD-induced obesity and hepatic steatosis in NAFLD rats. The results showed that the fermented hawthorn extract was effective in reducing body weight, treating HFD-induced NAFLD in rats.

In order to elucidate the mechanism of fermented hawthorn extract in treating NAFLD, we detected the mRNA and protein expression levels of CYP2E1, SREBP-1c and its target gene FAS in liver tissues, and observed the antioxidant effects of fermented hawthorn extract. Excess fatty acids in the liver can promote the expression of SREBP-1c and its downstream gene FAS in hepatocytes, leading to an imbalance of fatty acid metabolism, and increased TG synthesis, which can also up-regulate CYP2E1 expression, promote oxidative stress and superoxide production, causing damage to the hepatocytes [15]. The results of this study showed that high-dose fermented hawthorn extract markedly inhibited the mRNA and protein expression levels of CYP2E1, SREBP-1c and FAS in rat liver tissues. Through inhibition of CYP 2E1 expression in liver tissues of NAFLD rats, the lipid peroxidation reaction was reduced and the antioxidant capacity was enhanced, resulting in the improvement of hepatic steatosis and inflammatory damage in liver tissues. The MDA content in the liver is a biomarker of lipid peroxidation and oxidative stress. SOD and GSH are the key superoxide-scavenging

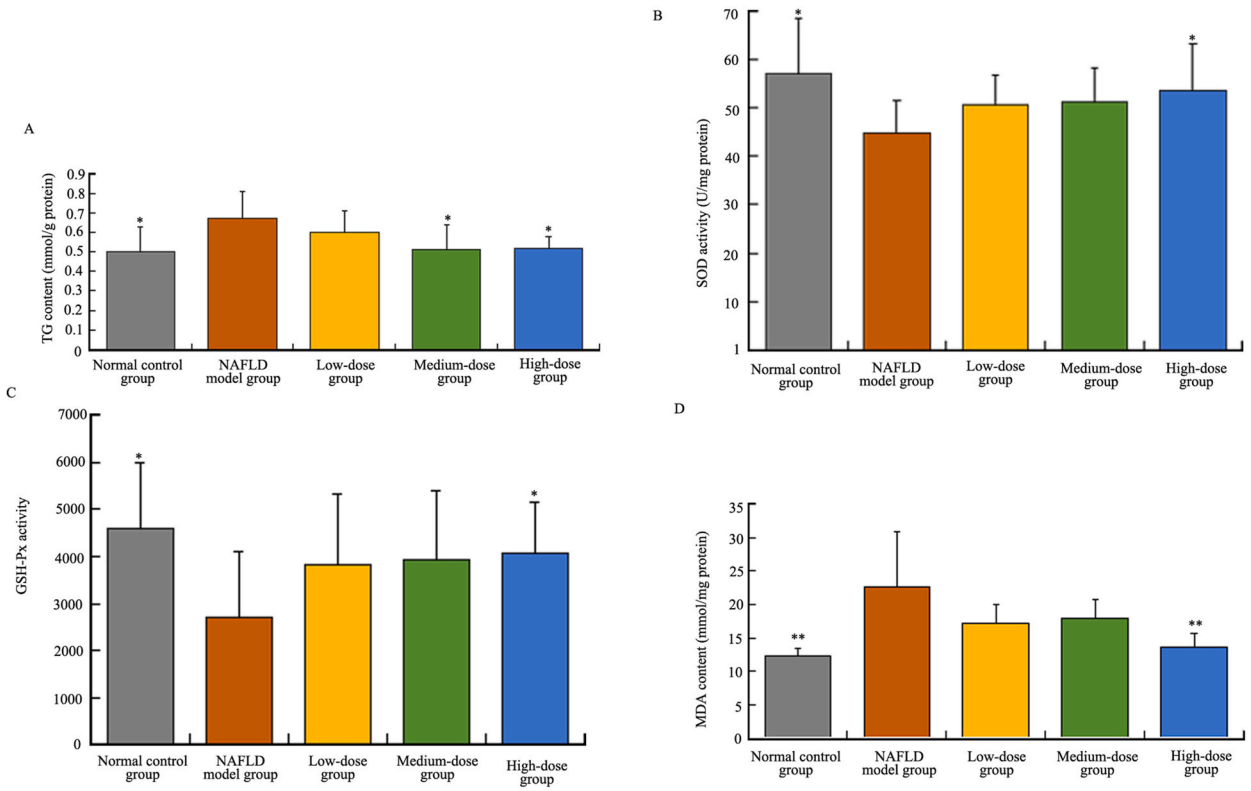

Fig. 5. Effect of fermented hawthorn extract on TG content, SOD, GSH-Px activities, and MDA content in liver tissues of rats with NAFLD. A: TG content; B: SOD activity; C: GSH-Px activity; D: MDA content. *$p < 0.05$, **$p < 0.01$, vs. the model group. NAFLD, non-alcoholic fatty liver disease; TG, triglyceride; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; MDA, malondialdehyde.

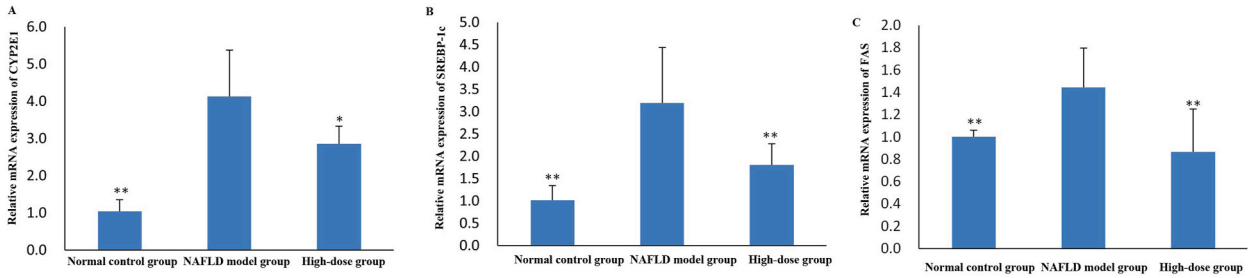

Fig. 6. High-dose fermented hawthorn extract inhibits the mRNA expression of CYP2E1 (A), SREBP-1c (B) and FAS (C) in liver tissues of NAFLD rats. *$p < 0.05$,**$p < 0.01$, vs the model group. NAFLD, non-alcoholic fatty liver disease; CYP2E1, cytochrome P4502E1; SREBP-1c, sterol regulatory element-binding protein 1c; FAS, fatty acid synthase.

enzymes, their activities can reflect the changes in the production and scavenging of free radicals. The results of this study also showed that treatment with fermented hawthorn extract significantly inhibited the MOD content and increased the activities of SOD and GSH in rat liver tissues. Fermented hawthorn extract also reduced TG accumulation in liver tissues in rat model of NAFLD. Our findings suggest that fermented hawthorn extract may exert its effect in treating NAFLD through suppressing the expression of SREBP-1c and its downstream gene FAS, inhibiting CYP2E1 expression, promoting the restoration of balance oxidative stress and antioxidant mechanisms, and reducing TG synthesis.

Hawthorn, as a Chinese traditional food for both medicinal and edible purposes, has good safety. The commonly recommended dose for adult usually exceeds 50–100g/day [16,17]. The high dose of fermented hawthorn extract used in this experiment is calculated to be equivalent to a dose of only 60g/day in adults. And the results revealed that medium- and high -dose fermented hawthorn extract significantly reduced the body weight in HFD-induced NAFLD rats, this finding is surprising, however, the exact mechanism of this effect needs to be further investigated. In this study, HFD caused the development of steatohepatitis in rats, although the body weight returned to normal after treatment, but the liver did not completely return to normal, suggesting that even though

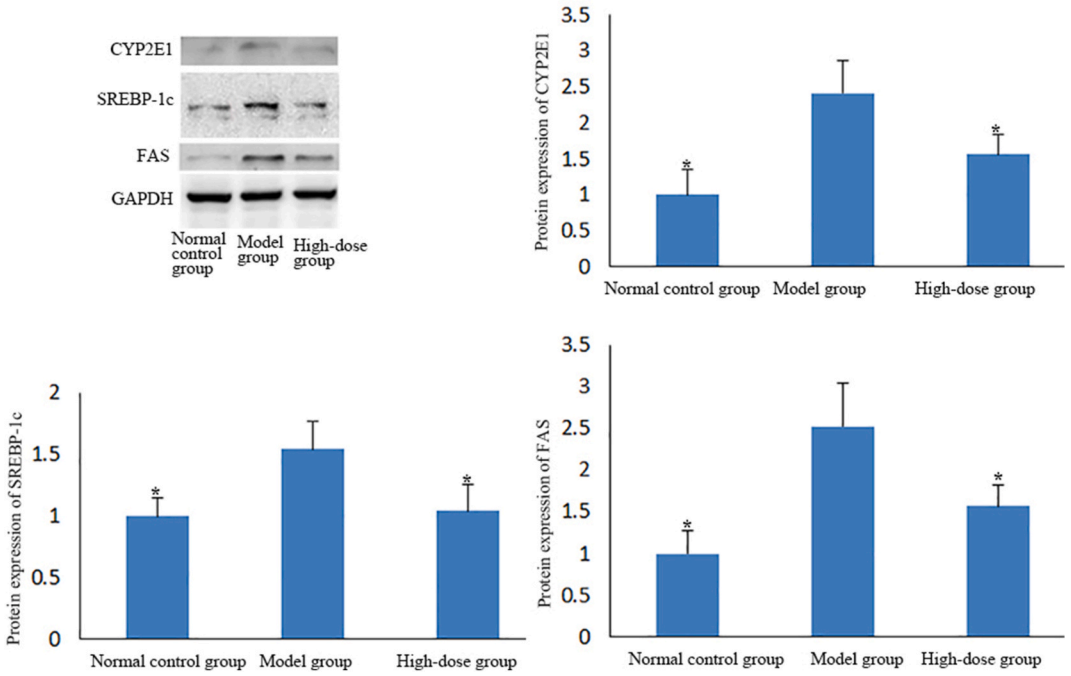

Fig. 7. High-dose fermented hawthorn extract inhibits the protein expression of CYP2E1, SREBP-1c and FAS in liver tissues of NAFLD rats. *$p < 0.05$,**$p < 0.01$, vs the model group. The raw blots of Fig. 7 is provided in Supplement Fig. 1. NAFLD, non-alcoholic fatty liver disease; CYP2E1, cytochrome P4502E1; SREBP-1c, sterol regulatory element-binding protein 1c; FAS, fatty acid synthase.

body weight can be strictly controlled to the normal range, it is difficult for the liver to return to normal without restricting the intake of high-fat foods.

The study also has some limitations. First, currently, there is no specific drug for NAFLD in modern medicine, so comparison between fermented hawthorn extract and modern drugs for the treatment of NAFLD cannot be performed. Second, this study did not identify major active pharmacological ingredients of fermented hawthorn extract. Furthermore, the possible signaling pathways by which fermented hawthorn extract exert its antioxidant effects are not explored in this study, which need further investigation.

Our findings suggest that fermented hawthorn extract could be used as a potential food for the treatment of NAFLD, which is worthy of clinical application and promotion, but further clinical studies are still needed.

Ethics statement

All animal procures were approved by the Animal Ethics Committee of Huazhong University of Science and Technology Union Shenzhen Hospital (No.ky-2018-010302).

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Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Zhiqiang Gao: Writing – original draft, Supervision, Formal analysis, Data curation. **Meijuan Xie:** Writing – review & editing, Resources, Formal analysis, Data curation. **Ruyun Zhou:** Writing – review & editing, Visualization, Methodology, Conceptualization. **Kaixin Wang:** Writing – review & editing, Software, Investigation, Data curation. **Jiang Li:** Writing – review & editing, Supervision, Resources, Formal analysis. **Juan Zhang:** Writing – review & editing, Validation, Software, Formal analysis. **Libo Chen:** Writing – review & editing, Supervision, Methodology, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e37354.

References

[1] E.E. Powell, V.W. Wong, M. Rinella, Non-alcoholic fatty liver disease, Lancet. 397 (10290) (2021) 2212–2224.
[2] X.J. Liu, X.K. Hu, H. Yang, L.M. Gui, Z.X. Cai, M.S. Qi, C.M. Dai, A review of traditional Chinese medicine on treatment of diabetic nephropathy and the involved mechanisms, Am. J. Chin. Med. 50 (7) (2022) 1739–1779.
[3] S. Liu, X. Chang, X. Liu, Z. Shen, Effects of pretreatments on anthocyanin composition, phenolics contents and antioxidant capacities during fermentation of hawthorn (Crataegus pinnatifida) drink, Food Chem. 212 (2016) 87–95.
[4] Z.Q. Gao, M.J. Xie, L.B. Chen, Effects of hawthorn berries, leaves on lipid metabolism and oxidative stress in fatty liver rats, Sichuan Zhongyi 34 (2016) 50–53.
[5] Z. Gao, M. Xie, N. Wang, L. Chen, X. Huang, Effects of combination treatment of metformin and hawthorn in patients with prediabetes complicated by nonalcoholic fatty liver disease, Int. J. Clin. Exp. Med. 12 (12) (2019) 1979–1984.
[6] Xi Che, J. Mao, S.P. Liu, Z.L. Zhou, J.B. Xue, Screening of monacolin K-producing monascus and effects of Chinese medicinal herbal materials on monacolin K production in solid-state fermentation, Shipin Kexue (Beijing) 37 (13) (2010) 114–119.
[7] W.W. Jing, Preliminary Study on Two-Way Fermentation System of Monascus, Guizhou: Guizhou University, 2009.
[8] W. Zhu, S. Chen, R. Chen, Z. Peng, J. Wan, B. Wu, Taurine and tea polyphenols combination ameliorate nonalcoholic steatohepatitis in rats, BMC Complement Altern Med 17 (1) (2017) 455.
[9] G.T. Brown, D.E. Kleiner, Histopathology of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis, Metabolism 65 (8) (2016) 1080–1086.
[10] P. Golabi, J.M. Paik, S. AlQahtani, Y. Younossi, G. Tuncer, Z.M. Younossi, Burden of non-alcoholic fatty liver disease in asia, the Middle East and north africa: data from global burden of disease 2009-2019, J. Hepatol. 75 (4) (2021) 795–809.
[11] B. Liu, G.H. Lv, Analysis of the rule of hawthorn in the treatment of fatty liver disease based on data mining, Zhongguo Minzu Minjian Yiyao. 29 (22) (2020) 8–11.
[12] S. Al Humayed, Protective and therapeutic effects of Crataegus aronia in non-alcoholic fatty liver disease, Arch. Physiol. Biochem. 123 (1) (2017) 23–30.
[13] J.Y. Yang, Y.Q. Lai, Y.X. Li, S.Y. Yang, X.R. Li, X.H. Huang, Preparation process of lipid-lowering medicines by solid state fermentation of Crataegi Fructus, Alismatis Rhizoma, and Cassiae Semen mixed with monascus, Chin. Tradit. Herb. Drugs 47 (12) (2016) 2100–21008.
[14] Y. Yan, C. Liu, S. Zhao, X. Wang, J. Wang, H. Zhang, Y. Wang, G. Zhao, Probiotic Bifidobacterium lactis V9 attenuates hepatic steatosis and inflammation in rats with non-alcoholic fatty liver disease, Amb. Express 10 (1) (2020) 101.
[15] J. Mun, S. Kim, H.G. Yoon, Y. You, O.K. Kim, K.C. Choi, Y.H. Lee, J. Lee, J. Park, W. Jun, Water extract of curcuma longa L. Ameliorates non-alcoholic fatty liver disease, Nutrients 11 (10) (2019) 2536.
[16] Z.G. Wen, M.S. Shao, L.H. Zhao, Study on the clinical application and dosage of hawthorn fruit, Changchun Zhongyiyao Daxue Xuebao. 37 (4) (2021) 741–744.
[17] G.H. Jiang, R.X. Chen, Hawthorn fruit, natural "Jianwei Xiaoshi tablet", Zhongyi Yangsheng Zazhi 7 (11) (2021) 30–31.