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Research article

Research on the mechanism of sea buckthorn leaf Fu tea in the treatment of hyperlipidemia

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ABSTRACT

Background: Hyperlipidemia (HLP) presents a significant challenge to global public health. Mounting evidence suggests that statins, the recommended first-line lipid-lowering agents, have significant adverse effects. Consequently, the quest for natural and efficacious alternative therapies is steadily emerging as a research priority for HLP prevention and treatment. Consumption of tea, which is rich in diverse biologically active compounds with the capacity to regulate lipid metabolism and combat obesity, has emerged as a promising alternative therapy. Sea buckthorn leaves are rich in a multitude of biologically active substances, have a hypolipidemic effect, and can be used as a raw material for tea because of their unique flavor. There is a suggestion that combining *Aspergillus cristatus* with tea could modify or boost the lipid-lowering active compounds present in tea, thereby increasing its efficacy in regulating lipid metabolism.
Results: Sea Buckthorn Leaf Fu Tea (SBLFT) was obtained by fermentation when sea buckthorn leaves contained 42 % moisture, inoculated with *Aspergillus cristatus* 0.2 mL/g, and incubated for 8 d at constant temperature. Animal experiments demonstrated that SBLFT significantly inhibited body weight gain in HLP rats and reduced lipid content and serum oxidative stress. In addition, liver tissue sections and functional indices showed that SBLFT can improve liver morphology and function abnormalities. Reverse transcription-polymerase chain reaction results indicated that the expression of Liver kinase B1 (*LKB1*), adenosine 5'-monophosphate (AMP)-activated protein kinase (*AMPK*), acetyl CoA carboxylase 1 (ACC1), and sterol-regulatory element binding protein-1 (*SREBP1c*) gene related to lipid metabolism was altered.
Conclusion: SBLFT improved HLP, specifically via promoting the expression of *LKB1* in the liver of HLP rats, activating *AMPK*, and inhibiting *ACC1* and *SREBP1c* expression, resulting in the inhibition of fatty acid and triglyceride synthesis-related enzymes at the transcriptional level.

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1. Introduction

Hyperlipidemia (HLP) is a condition in which abnormalities in fat metabolism or function cause blood lipid levels to exceed the normal range, including elevated levels of total cholesterol (TC), triglycerides (TG), and low-density Lipoprotein Cholesterol (LDL-C), and decreased levels of high-density lipoprotein cholesterol (HDL-C) [1]. HLP, caused by abnormal lipid metabolism, is a serious threat to human health and quality of life, and a key risk factor for cardiovascular diseases [2]. Statins are the main drugs used to treat HPL; however, they have many limitations, including drug resistance, drug intolerance due to adverse effects, and lack of patient compliance [3]. Therefore, natural medicines or functional foods with no toxic side effects are being developed as a supplement or alternative to statins. People favor dietary supplements that improve lipid metabolism; among the many options, tea is preferred by most people [4].

Tea is a popular beverage made from the leaves of the tea plants and is popular worldwide because of its taste and richness in various active substances [5]. Tea is rich in polyphenols, polysaccharides, and alkaloids, which play an important roles in the regulation of cardiovascular diseases, cancer, diabetes, and other diseases [6]. In Addition, tea and the its active ingredients also play an important role in the fight against obesity. Epidemiological studies have demonstrated that individuals with a prolonged history of tea consumption exhibit lower body fat levels than non-tea drinkers. Experimental studies have indicate that tea consumption can effectively reduce weight and improve obesity, primarily due to increased energy expenditure and the promotion of lipolysis [7].

Tea is divided into fermented and non-fermented tea according to whether fermentation occurs during the production process. Black teas are fermented, whereas white and green teas are not fermented [8]. Black tea is produced through a series of steps after microbial fermentation, Previous lipid-lowering experiments of fermented and unfermented teas in animals have shown that fermented teas lower the levels of blood lipids and cholesterol [9]. Daily consumption of a type of fermented tea, Fu Brick Tea (FBT) can reduce blood lipid levels in humans or experimental animals and has a certain effect on weight loss. The microorganism required to make FBT is *Aspergillus cristatus*, also known as 'golden flowering bacterium' because of its bright yellow color during fermentation. The fermentation process is key to improving the taste of FBT, and adding or altering the original ingredients can increase its benefits. Owing to its superior characteristics, *A. cristatus* has become the quality standard for FBT, and its has been demonstrated to be safe and non-toxic in food products [10]. Moreover, direct administration of *A. cristatus* has been shown to improve obesity and lipid metabolism abnormalities in mice fed a high-fat diet [11].

Sea buckthorn (*Hippophae rhamnoides* L.) is a plant of in the family of sea buckthorn, native to Europe and Asia. The majority of the sea buckthorn plants grow in China, Northern Europe, and Mongolia. Sea buckthorn leaves are rich in flavonoids, which are effective in preventing obesity, dyslipidemia, atherosclerosis, and antioxidants, The plants have a wide habitat, grow in large number, and rich in active substances, that play a crucial role in the nutritional and health care [12]. Although sea buckthorn leaves contain a variety of active substances and pharmacological effects, research on the whole sea buckthorn plant has mostly focused on sea buckthorn fruits, which have been demonstrated to improve lipid metabolism [13] and lower blood glucose [14]. However, the leaves of the sea buckthorn have been less studied, and most sea buckthorn leaves are disposed of as waste after the sea buckthorn fruit harvest, which does not utilize this high-quality plant resource, resulting in environmental and resource waste.

Based on the above studies, the purpose of the current study was to explore the potential mechanism of sea buckthorn leaf Fu tea (SBLFT) in the treatment of HPL using sea buckthorn leaves fermented by *A. cristatus* in a rat model of HPL.

2. Materials and methods

2.1. Materials and instruments

A. cristatus was purchased as a component of Jingyang FBT (Jingwei Fu Tea Company Limited, Xianyang, Shaanxi Province Shaanxi, China), and selected and purified in the laboratory to obtain the pure strain of *A. cristatus*. Sea buckthorn leaves were purchased from Ningxia Vanilla Biotechnology Company Ltd. (Ningxia, China). Lipid metabolism, including TG, TC, HDL-C, and LDL-C; liver panel including AST and ALT; oxidation-reduction balance, including SOD, GSH-Px, MDA, and hematoxylin-eosin (HE) test kits were purchased from Nanjing JianChen Bioengineering Institute (Nanjing, China). Total RNA was extracted from the cells using TakaRa Bio kits (Beijing, China). All other chemical reagents were obtained from Tianjin Tianli Chemical Reagent Co., Ltd. (Tianjin, China). A WPL-125BE constant-temperature incubator was obtained from Tester Instruments (Tianjin, China). The YX-24LDJ pressure and steam sterilizer were obtained from Jiangyin Binjiang Medical Equipment Company, Ltd (Jiangsu, China). The thermoenzyme markers were purchased from Thermo Fisher Scientific (USA).

2.2. Acquisition and identification of Aspergillus cristatus

Golden *A. cristatus* was obtained from Jingyang FBT, inoculated on potato dextrose agar (PDA) solid medium, and streaked for isolation 4–5 times. Mycelial coverslips from different periods were obtained using the insertion method and placed under a microscope to observe microscopic features. Mycelia was subjected to liquid culture, collected and DNA was extracted. Reverse transcription-polymerase chain reaction (RT-PCR) was used to amplify fungal ITS sequences (GeneBank lookup, Primer 5.0 design) with primer sequences of ITS1 sequence (TCCGTAGGTGAACCTGCGG) and ITS4 sequence (TCCTCCGCTTATTGATATGC). After purification of the target fragments, the T vector was ligated and sequencing was performed by Shanghai Sangong Bioengineering Company Limited (Shanghai, China). The sequencing results were compared in the NCBI database using the Blast tool.

2.3. Process study of SBLFT

A. cristatus liquid ferment was inoculated on clean sea buckthorn leaves. The temperature and humidity conditions of the incubator were controlled to set the fermentation time (4, 6, 8, and 10 d) and water content of sea buckthorn leaves (25, 35, 45, and 55 %). The amount of inoculum (0.05, 0.1, 0.15, and 0.2 mL/g) and the single variable method were used to conduct the univariate Experiment. The response surface design and optimization were then performed using the Minitab software. *A. cristatus* spore quality was used as the selection index. The spores were observed and photographed under a light body microscope, and 60–80 Aurelia spores were labeled with a scale using the ruler tool in PhotoShop software to calculate the spore size.

2.4. Animals and diet

This study fully complied with the rules for management of experimental animals issued by the Animal Ethics Committee of Shaanxi University of Chinese Medicine (Shaanxi, China). Animal Ethics Number: SUCMD20210303001. All the experiments were performed in compliance with the Guidelines on Laboratory Animal Management (English version: ISBN-10: 0-309-15396-4) issued by the National Science and Technology Commission (China). Seventy SPF-grade male Wister rats, with an average weight of 200 ± 10 g, were procured from BeiJing Huafukang Biological Science and Technology Company Limited (Beijing, China). The license number for this company is SCXK (Beijing) 2019-0008. The rats were kept in separate cages in the Standard Animal Laboratory of Clinical Chinese Medicine at Shaanxi University of Chinese Medicine under controlled conditions (18–24 °C, humidity 45 % ± 5 %, and 12 h light/dark cycle).

After 5 days of acclimatization, all rats were divided into six groups of 10 rats each as follows: blank control (NC), high-fat model (HLM), positive drug simvastatin (SV), SBLFT low-dose (SBLFT-L), SBLFT medium-dose (SBLFT -M) and SBLFT high-dose (SBLFT-H). Rats in the NC group were fed normal chow and orally administered 2 mL of sterile water once per day for 9 weeks. Rats in the HLM were fed normal chow and administered high-fat emulsion (1 mL/100 g d) and sterile water by gavage once per day for 9 weeks. Rats in the SV group were fed normal chow and were administered high-fat emulsion (1 mL/100 g d) and simvastatin sterile aqueous suspension (2.1 mg/kg) by gavage daily for 9 weeks. The SBLFT-L, SBLFT-M and SBLFT-H groups were fed normal feed and administered high-fat emulsion (1 mL/100 g d) and sterile water-extracted SBLFT solution (1.6 g/mL, 3.2 g/mL and 4.8 g/mL, respectively) by gavage once per day for 9 weeks. All rats were given free access to water and food, and their mental status and dietary intake monitored and documented daily. Weight and body length (from the snout to the anus) were measured weekly. The high-fat emulsion was prepared by dissolving 10 g of cholesterol and 1 g of propylthiouracil in 25 g of preheated lard, followed by the addition of 25 mL of Tween-80 and thorough mixing. In addition, 2 g of sodium cholate were weighed and dissolved in a mixture of 30 mL of distilled water and 20 mL of propylene glycol preheated to 60 °C. The emulsion and solution were then combined to obtain the high-fat emulsion required for the experiment. The aqueous extract of SBLFT was prepared by mixing SBLFT with distilled water at a ratio of 1:10, boiling for 30 min, and filtering to obtain the aqueous extract of SBLFT. The remaining SBLFT was mixed with distilled water in the same proportion and boiled for 15 min, resulting in a second aqueous extract of SBLFT. The two SBLFT aqueous extracts were mixed and concentrated under reduced pressure to the corresponding concentration to obtain the target product.

2.5. Sample collection and measurement of blood biochemical indices

After the final SBLFT treatment, all rats were fasted for 12 h. Rats were euthanized by intraperitoneal injection of sodium pentobarbital and blood collected from the abdominal aorta. Blood samples were collected in sterile centrifuge tubes, centrifuged at 3000 rpm for 10 min, and the separated rat serum was stored at −80 °C for future use. Liver tissue and visceral fat (including epididymal and perirenal fat pads) were excised, 1/3 of the removed liver tissue was fixed in 10 % formalin, and the remaining portion was placed in sterile centrifuge tubes and stored at −80 °C for future use. The removed adipose tissues were weighed using an electronic balance to obtain their wet weight, after which they were stored in −80 °C for future use. Rat serum levels of outcome measures were determined using commercial kits in accordance with the manufacturer's instructions.

2.6. Histopathologic testing of the liver

Rat liver tissues fixed in 10 % formalin were removed and subjected in paraffin to a thickness of 4 μm, followed by HE staining [15]. The stained areas were observed using a light microscope and histopathological images of rat livers were examined and analyzed.

2.7. RT-PCR

PCR primer sequences were designed and synthesized using the NCBI database query, Beacon Designer 8.14 software proportioning, and Auqo Dingsheng Company. An adequate quantity of liver tissue was collected and RNA extraction was performed according to the instructions provided with the TaKaRa RNA Extraction Kit. Subsequently, the RNA concentration in each sample was determined. Finally, reagents were added in accordance with the manufacturer's instructions, followed by reverse transcription and quantitative fluorescence analysis. The primer sequence information is provided in Supplementary Table 1.

2.8. Data analysis

All data were analyzed using SPSS 24.0 statistical software and the results were expressed as mean ± standard deviation (SD). Multiple comparisons between groups were performed using one-way analysis of variance (ANOVA) test. "#" indicates a statistical difference at the $p < 0.05$ level compared with the NC group, and "*" indicates a statistical difference at the $p < 0.05$ level compared with the HLM group.

3. Results

3.1. Molecular biological characterization of A. cristatus

Before the experiment, we identified, isolated, and purified *A. cristatus* from Jingyang FBT to ensure that it was the strain needed to produce SBLFT. Purified *A. cristatus* was detected using agarose gel electrophoresis. The amplified band was approximately 600 bp, which was the same size as the predicted fragment and was initially identified as the desired target gene. Subsequent comparison with the database showed a 98 % similarity to *A. cristatus* (Sequence ID: AB002073.1) (Supplementary Fig. 1).

3.2. SBLFT fabrication

The quality of the SBLFT is closely related to the size of the *A. cristatus* spores. Spore size is related to the time of SBLFT fermentation, moisture content of sea buckthorn leaves, and amount of inoculation, therefore, it is necessary to strictly control the above influencing factors during preparation to obtain better quality SBLFT.

Based on the results of the single-factor experiment (Supplementary Fig. 2), the moisture content of 35–45 % and the inoculum amount of 0.15–0.2 mL/g were finally selected as the optimal settings, and the fermentation time was 8 days. The quadratic multiple regression fit to the measured spore size values (Supplementary Table 2) yielded a quadratic multiple regression equation of spore size (Y), moisture content (X_1), and inoculum (X_2) (Eq. (1)).

$$Y = -1278.9 + 92.39X_1 + 408X_2 - 0.7659X_1^2 - 1115X_2^2 + 4.98X_1X_2 \quad (1)$$

The model was significant ($p < 0.05$), with highly significant ($p < 0.001$) X^1, X^2, and X_1^2, adjusted $R^2 = 0.9962$, and predicted $R^2 = 0.982$ (Supplementary Fig. 3A). The F-test for the significance of the multiple regression equation and misfit exhibited high significance ($p < 0.01$). This suggests the significance of the response surface simulation and validates the feasibility of utilizing this equation for two-factor, two-level experiments. Response optimization was performed in Minitab software, and the results as shown in Supplementary Fig. 3B. The inoculum exhibited a linear positive correlation with the size of spores, and there was an extreme value

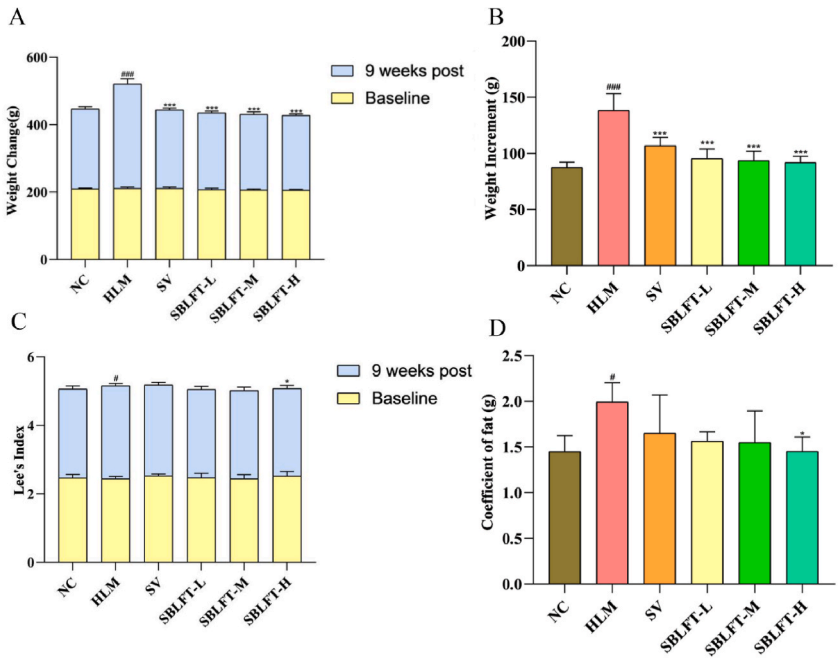

Fig. 1. Effect of SBLFT on body weight, Lee's index and coefficient of fat in rats. Fig. A–D: Changes in body weight, Lee's index and adiposity coefficient of rats before treatment, respectively. Compared with NC group: $\#P < 0.05$, $\#\#\#P < 0.001$. Compared with HLM group: $*P < 0.05$, $***P < 0.001$.

between 35 % and 45 % of the moisture content, The size of spores could reach 69.36 μm when the moisture content was 41.38 % and the inoculum amount was 0.2 mL/g, (95 % confidence interval of 67.071, 71.639), indicating the optimal condition for treatment. Contour inscriptions of moisture content (X_1) and inoculum (X_2) as well as surface 3D maps using spore size (Y) as the response value, gave the same results (Supplementary Fig. 3C\D). The experiments were validated under the optimal conditions of the treatment by choosing a moisture content of 41.5 %, an inoculum volume of 0.2 mL/g, and leaving all the remaining conditions unaltered, three parallel experiments were conducted and results averaged. Under these conditions, the *A. cristatus* were large and full, exhibiting a golden yellow color and uniform distribution, with a size of the spores of 71.46 μm.

3.3. Results of animal experiments

3.3.1. Effect of SBLFT on body weight and adiposity of rats

There was no statistical difference ($p > 0.05$) in the initial mean body weights of the rats after grouping, indicating consistency with the correct randomized grouping method (Fig. 1A). Compared to the NC group, the body weight of rats in the HLM group increased significantly ($p < 0.01$). Conversely, the body weights of rats in the other four administered groups displayed a decreasing trend and a significant difference between the high-fat model group and the HLM, ($p < 0.001$) (Fig. 1B). Lee's index is similar to human body mass index BMI, which mainly reflects the degree of obesity in an organism. The adiposity coefficient represents the percentage of fat pads surrounding the kidneys and testes relative to the weight of the rat. Its magnitude also indicates the level of obesity in rats. Lee's index and adiposity coefficients of rats in the SBLFT-H group had significantly lower values than those in the HLM group ($p < 0.05$), Additionally, Lee's index and adiposity coefficients of the other three groups were lower than those of the HLM group, although the differences were not statistically significant ($p > 0.05$) (Fig. 1C/D). The results of the above experiments showed that SBLFT intake effectively inhibit the abnormal increase in body weight and the degree of obesity in HLP rats, and the reduction in body weight and the change in the adiposity coefficient were dose-dependent. The SBLFT-H demonstrated the most significant improvement in body weight, Lee's index, and adiposity coefficient in the rats compared to the other groups.

3.3.2. Effect of SBLFT on blood lipids in rats

The levels of TG, TC and LDL-C in the HLM group were significantly higher than those in the NC group ($p < 0.001$) (Fig. 2 A/B/D), whereas the level of HDL-C was significantly lower ($p < 0.001$) (Fig. 2C), indicating that the presence of dyslipidemia in rats. Compared to the HLM group, the positive drug SV group, SBLFT-L, SBLFT-M, and SBLFT-H groups showed reduce levels of TG, TC, and LDL-C ($P < 0.001$) (Fig. 2 A/B/D) and elevate HDL-C levels ($P < 0.001$) (Fig. 2C). Furthermore, high doses of SBLFT were associated with comparable potency to improve TG, TC and LDL-C as the positive drug SV. In addition to that, SBLFT exhibited a dose-dependent trend in increasing TG, TC and LDL-C levels while decreasing HDL-C levels (Fig. 2).

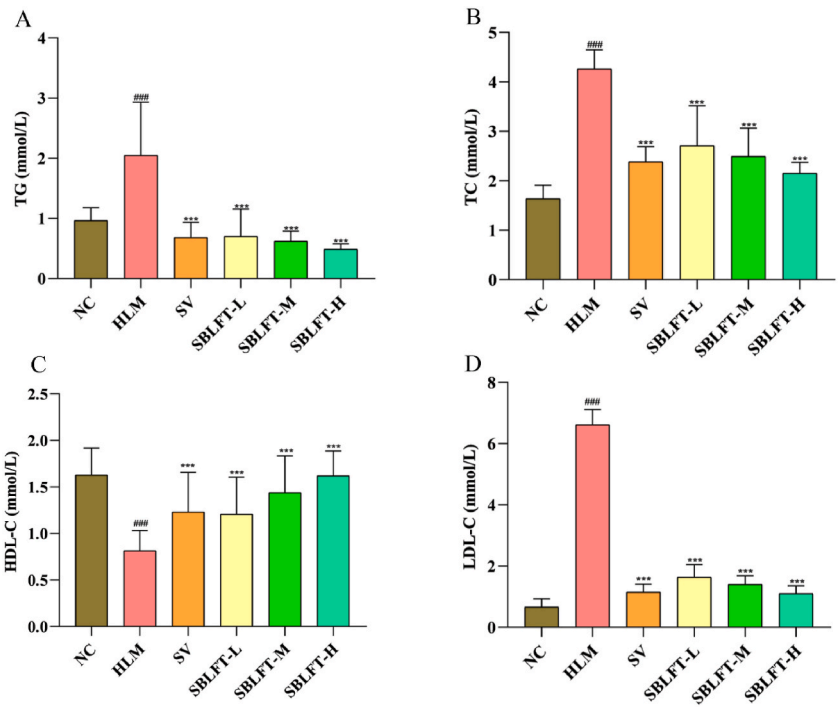

Fig. 2. Effect of SBLFT on serum lipid levels in rats. A–D: Levels of TG, TC, LDL-C and HDL-C in rats, respectively. Compared with NC group: ###$P < 0.001$. Compared with HLM group: ***$P < 0.001$.

3.3.3. Effect of SBLFT on rat liver tissue

The structure of the liver lobules of rats in NC group was clear, the hepatocytes were radially distributed around the central vein as the center, the size was uniform, the hepatic sinusoids were clearly visible, the hepatic cords were neatly arranged, the nuclei of the cells were clear, the cytoplasm was uniform, there no production of lipid droplets was present. Overall, the cellular morphology and structure were normal. The morphology and structure of hepatocytes of rats in the HLM group were clearly abnormal, the cell boundaries were unclear, the hepatocytes were loosely connected in a round shape, the swelling was severe, the structure of the liver lobules was disorganized, the hepatic sinusoids were shrunken or even disappeared, the hepatic cords were disorganized, and the lesion area was most evident in the area around the central vein. The presence of diffuse round white vacuolated lipid droplets in the cytoplasm of the cells, which were lightly stained and squeezed the nuclei to one side, indicated heavy steatosis. The above results indicate that gavage of high-fat emulsions induced lipid accumulation in rat liver tissue After the intervention by SBLFT, the lesions in the liver tissues of rats in all groups were improved, particularly in the SBLF-H group (Fig. 3).

3.3.4. Effect of SBLFT on liver function in rats

ALT and AST are often used to assess the severity of liver tissue damage. Serum levels of ALT and AST were significantly higher in the HLM group than in the NC group ($P < 0.001$) (Fig. 4 A/B), indicating that the liver was damaged by long-term gavage with high-fat emulsions. Compared with the HLM group, ALT and AST levels were significantly lower in the positive drug SV, SBLF-L, SBFL-M, and SBLF-H groups ($P < 0.001$) (Fig. 4 A/B), concurrently, SBLFT exhibited a more effective amelioration of ALT and AST compared to SV, although the difference was not statistically ($P > 0.05$) (Fig. 4 A/B). The results indicate that SBLFT significantly improve the levels of ALT and AST in HLP rats in a dose-dependent manner (Fig. 4).

3.3.5. Effect of SBLFT on serum oxidative stress levels in rats

Serum levels of SOD and GSH-Px were significantly lower ($P < 0.001$) (Fig. 5 A/C) and MDA levels were significantly higher ($P < 0.001$) (Fig. 5 B) in the HLM group than in the NC group, indicating that the HLM group was under oxidative stress. The levels of SOD and GSH-Px were significantly higher ($P < 0.001$) (Fig. 5 A/C), whereas the MDA levels were significantly lower ($P < 0.001$) (Fig. 5 B) in the SV, SBLFT-L, SBLFT-M, and SBLFT-H groups than in the HLM group. The results indicate that both SV and SBLFT improved the level of oxidative stress induced by high fat emulsion in HLP rats. For improvements in MDA and GSH-Px, SBLFT tended to be higher than the positive drug SV, although the difference was not statistically significant ($P > 0.05$) (Fig. 5 B/C). In addition, SBLFT improved of SOD, MDA and GSH-Px in a dose-dependent manner (Fig. 5).

3.3.6. Effect of SBLFT on LKB1, AMPK, SREBP1c and ACC1 mRNA levels in rat liver tissue

The expression levels of *LKB1* and *AMPK* mRNA in the livers of rats in the HLM group were significantly lower ($P < 0.001$) (Fig. 6 A/B) and the expression levels of *SREBP1C* and *ACC1*mRNA in the HLM group were significantly higher ($P < 0.001$) (Fig. 6C/D) than those in the NC group. The expression levels of *LKB1* and *AMPK* mRNA were significantly higher ($P < 0.001$) (Fig. 6 A/B), whereas the expression levels of *SREBP1C* and *ACC1* mRNA were significantly lower ($P < 0.001$) (Fig. 6C/D) in the livers of rats in the SV group than those in the HLM group, as well as the SBLF-L, SBLF-M, and SBLF-L groups. The RT-PCR results demonstrated that the positive drug SV and SBLFT significantly upregulated the expression levels of *LKB1*mRNA and *AMPK* mRNA and downregulating the expression levels of *SREBP1C* and *ACC1* mRNA in the livers of HLP rats, thereby ameliorating HLP. In addition, the SBLFT-H improved the expression of these genes comparable to the positive drug SV in a dose-dependent manner (Fig. 6).

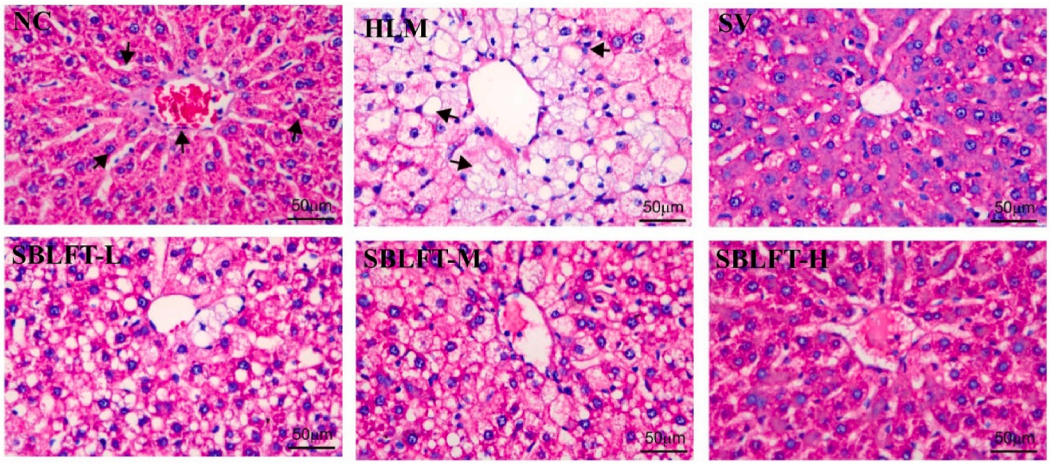

Fig. 3. HE staining pictures of rat liver tissue in each group. NC: HE staining of rat liver in NC group. HLM: HE staining of rat liver in HLM group. SV: HE staining of rat liver in SV group. SBLF-L: HE staining of rat liver in SBLF-L group. SBLF-M: HE staining of rat liver in SBLF-M group. SBLF-H: HE staining of rat liver in SBLF-H group.

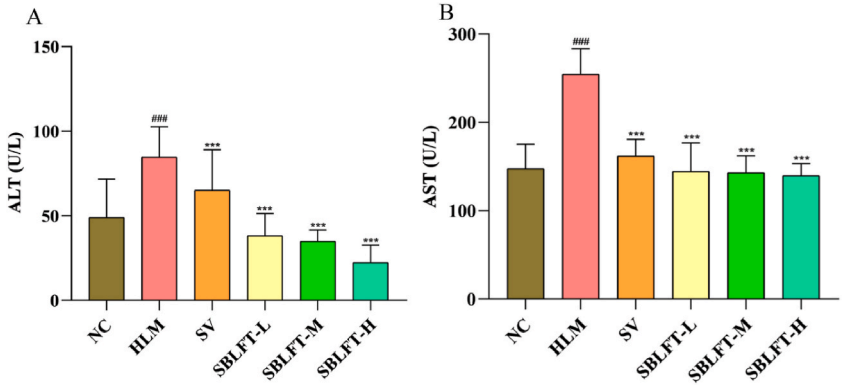

Fig. 4. Effect of SBLFT on liver function in rats. A–B: Changes of ALT and AST in rat serum, respectively, compared with NC group: $###P < 0.001$. Compared with HLM group: $***P < 0.001$.

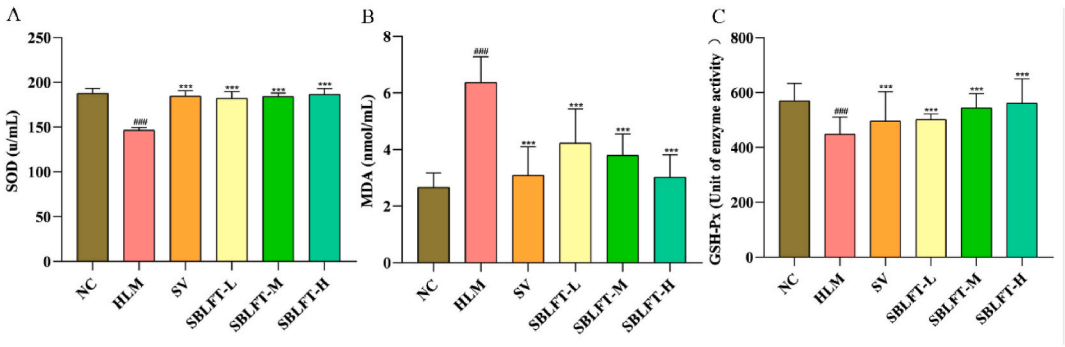

Fig. 5. Effect of SBLFT on oxidative stress indices in rat serum. A–C: Serum levels of SOD, MDA and GSH-Px in rats, respectively. Compared with NC group: $###P < 0.001$. Compared with HLM group: $***P < 0.001$.

4. Discussion

The diagnostic criteria for HLP, a common disease in the modern society, include a significant deviation in one or more plasma lipids from the normal range, including elevated TG, TC, and LDL-C, as well as reduced HDL-C levels [3,16]. As the human body is rich in the most abundant lipids, when the blood TG level is elevated, TG is deposited on the walls of blood vessels, causing blood vessel occlusion and atherosclerosis. TC, as a measure of dyslipidemia, is a key indicator of abnormalities, and abnormal changes in LDL-C and HDL-C indicate abnormal lipid metabolism in the body [17–21]. In this experiment the plasma lipids of rats gavaged with high-fat emulsion showed significant abnormalities compared with the NC group, which reached the diagnostic criteria of hyperlipidemia, indicating that the rats suffered from HLP. SBLFT effectively normalizes the plasma lipid of rats with HLP. This indicates that SBLFT can effectively treat HLP by improving diagnostic indicators.

The liver plays a crucial role in coordinating lipid metabolism within the body, and abnormal lipid metabolism can result in impaired liver function, which in turn can further disrupt lipid metabolism. These processes mutually influence each other [22,23]. ALT and AST are important indicators of liver function. When there is damage to the structure or function of the liver, ALT and AST levels in the blood significantly exceed the normal ranges [24,25]. In the present experiment, administration of high-fat emulsion increased the levels of AST and ALT in rats, and SBLFT was effective in reducing the levels of AST and ALT. SBLFT as a drug alternative must be examined for its safety. The experimental results showed that SBLFT had no toxic side effects on the rat liver. Observation of rat liver sections revealed that administration of SBLFT not only did not induce liver damage in animal models but also alleviated liver damage associated with HLP. However, as an alternative drug product, it is necessary to further observe the safety and therapeutic efficacy of SBLFT in populations wit hyperlipidemia.

Dyslipidemia induces lipid peroxidation in the body, which can also cause hyperlipidemia [26,27]. SOD, CAT, and GSH-Px as endogenous antioxidant enzymes in the human body that possess the capability to enhance oxidative status in vivo. SOD scavenges free radicals, whereas GSH-Px scavenge hydrogen peroxide and lipid peroxides [28,29]. MDA is a product of lipid oxidation and is formed as a result of free radical attack on plasma membrane proteins and polyunsaturated fatty acids [30]. In this experiment, SBLFT effectively increased the serum levels of SOD and GSH-Px and reduced MDA in HLP rats. These results suggest that SBLFT can alleviate oxidative stress and potentially treat HLP.

AMPK is a serine/threonine protein kinase highly conserved in multiple metabolic pathways, and is composed of a catalytic subunit

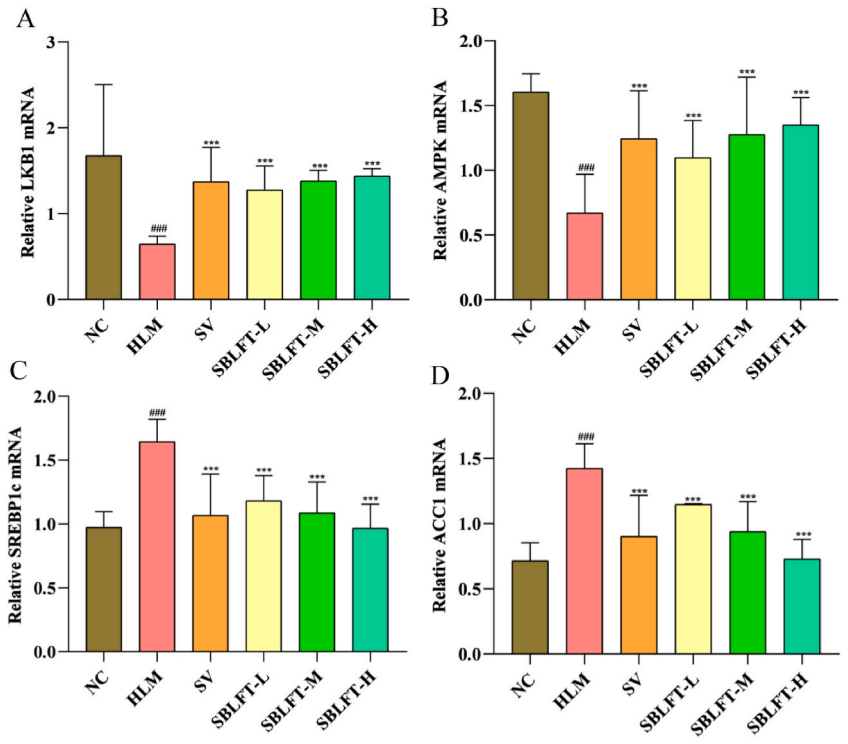

Fig. 6. Expression of LKB1, AMPK, SREBP1c and ACC1 mRNA in rat liver. A–D: Expression of LKB1, AMPK, SREBP1c and ACC1 mRNA in rat liver tissues, respectively. Compared with the NC group: $###P < 0.001$; compared with the HLM group: $***P < 0.001$.

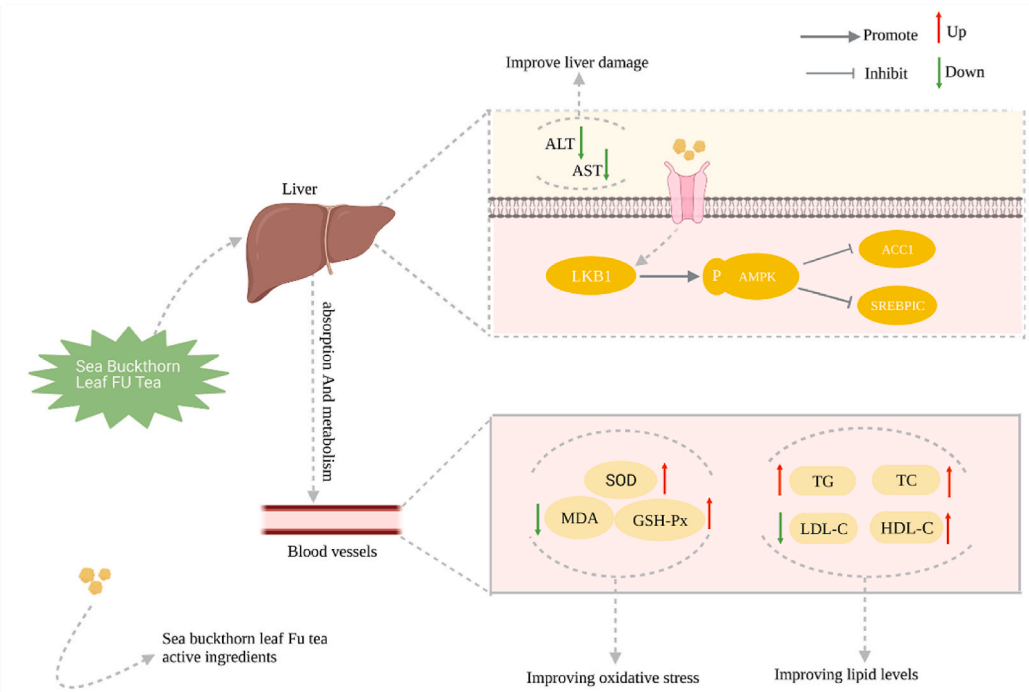

Fig. 7. Mechanisms by which SBLFT improves HLP.

α and two regulatory subunits β and γ. As a central regulator of multiple metabolisms, AMPK regulates energy metabolism in the body [31]. Similarly AMPK plays a key role in the regulation of hepatic lipid metabolism [32,33]. AMPK activation involves complex and diverse mechanisms that are intricately linked to the energy status of mammalian cells. This status is governed by the AMP/ATP ratio; an increase in this ratio activates AMPK, whereas a decrease diminishes its activity [34]. AMPK activity is also affected by the upstream regulatory kinases LKB1 and (calmodulin-dependent protein kinase) CaMKKβ, which enhance the level of phosphorylation of AMPK by promoting phosphorylation of the Thr172 site on the AMPKα subunit, leading to AMPK activation [35]. The LKB1/AMPK pathway is important for lipid metabolism, and it has been shown that activation of the LKB1/AMPK axis prevents liver disease [36,37]. AMPK activation initiates regulatory effects on lipids via diverse signaling pathways. AMPK can hinder ACC1 expression, a key contributor to fatty acid synthesis. This inhibition occurs through AMPK phosphorylation at Ser 2 and Ser 79 of ACC1, thereby suppressing its activity [38]. AMPK phosphorylation also inhibits the expression of fatty acid and TG synthesis-related enzymes and cholesterol metabolism at the transcriptional level by suppressing SREBP1c [39]. SREBP1c serves as a pivotal regulator of hepatic lipid metabolism, is synthesized within the endoplasmic reticulum of the cells, and governs the maturation of SREBP1c in the endoplasmic reticulum. Upon maturation, SREBP-1c translocates to the nucleus to activate adipogenic genes. Additionally, the expression of SREBP-1c is strongly linked to the enzyme ACC, which regulates fatty acid synthesis rates [40,41]. In the present study, the expression levels of rat liver-related genes were determined by RT-PCR. The experimental results showed that SBLFT significantly elevated the expression of *LKB1* and *AMPK* mRNA and decreased the expression of *SREBP1c* and *ACC1* mRNA in the liver tissue of hyperlipidemic rats. This indicates that SBLFT may inhibit fat synthesis and lipid accumulation in liver tissues of hyperlipidemic rats by regulating the AMPK/SREBP1c pathway, thus improving HLP.

5. Conclusion

In summary, *A. cristatus* was used to ferment sea buckthorn leaves to fabricate SBLFT, which exhibited lipid-lowering properties. The conditions required for SBLFT production were determined by examining the factors involved in SBLFT fermentation, which may aid in the industrial production of SBLFT. In addition, SBLFT exerted a notable impact on lipid metabolism in HLP rats. This effect was evidenced by the direct improvement of blood lipids, liver health and function, reduction of in vivo oxidative stress, and modulation of gene expression associated with lipid metabolism in the rat liver. Finally, SBLFT a black tea with excellent lipid-lowering properties may play a role in improving HLP and alleviating obesity epidemic in the future.

Figures illustrate

Fig. 1 illustrates the improvement of SBLFT on body weight, Lee's index and coefficient of fat in rats.
Fig. 2 illustrates the improvement of lipid metabolism-related indices in rats by SBLFT.
Fig. 3 illustrates the improvement in rat liver cell morphology by SBLFT.
Fig. 4 illustrates the improvement of liver function indices in rats by SBLFT.
Fig. 5 illustrates the improvement of oxidative stress levels in rats in vivo by SBLFT.
Fig. 6 illustrates the improvement of SBLFT on mRNA levels related to lipid metabolism in rat liver.
Fig. 7 illustrates the specific reasons and corresponding mechanisms of SBLFT for improving HLP in rat.

Ethics statement

In this study, all animal studies fully complied with animal ethics Committee of Shaanxi University of Chinese Medicine (Shaanxi, China). Animal Ethics Number: SUCMD20210303001. and all data are authentic.

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Data availability statement

Study-related data are not deposited in publicly available repositories and the authors are not authorized to share the data. If data are required please obtain them from the corresponding author.

CRediT authorship contribution statement

Si-Kai Chen: Writing – original draft. **Wen-Xin Wei:** Investigation. **Feng-Yu Huang:** Software. **Jing Wang:** Conceptualization. **Xing-Yu Li:** Investigation. **Yu-Ting Yang:** Software. **Wan-Tao Xing:** Software. **Feng Gao:** Methodology, Funding acquisition. **Min Li:** Resources. **Feng Miao:** Writing – review & editing. **Liang-Liang Chen:** Writing – review & editing, Supervision. **Pei-Feng Wei:**

Writing – review & editing, Project administration, Methodology, Investigation, Funding acquisition.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e32343.

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