Protective Effect of Sea Buckthorn (Hipphophae Rhamnoides) Extract on Liver Injury Induced by High-Fat Diet in Mice

Si SUN, Bingqing LI, Shuang PENG, Lian HE

College of Basic Medicine, Changsha Medical University, Changsha, Hunan 410219, China

Abstract: This experiment aimed to investigate the protective effect of sea buckthorn (Hipphophae rhamnoides) extract on an animal model of NAFLD induced by high-fat and cholesterol diet. Twenty-five SPF-grade male KM mice were randomly divided into the blank control group, high-fat model group, sea-buckthorn low-dose group, sea-buckthorn medium-dose group, and sea-buckthorn high-dose group. During the whole experiment, the high-fat model group and sea-buckthorn treatment group were fed high-fat and high-cholesterol diet to build the fatty liver model, whereas the blank control group was fed ordinary diet. The high-fat model group and blank control group were intragastrically given normal saline, and each sea buckthorn treatment group was intragastrically given different concentrations of sea buckthorn extract. After 5 weeks of intervention using the abovementioned method, the experiment was completed; relevant serological indexes were determined, and the liver coefficient was calculated. Our results demonstrated that the liver coefficient in the high-dose sea buckthorn group was extremely significantly decreased \((P < 0.01)\) compared with that in the high-fat model group. In addition, the concentration of total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) in serum of mice was decreased by the intervention of sea buckthorn extract, whereas the concentration of high-density lipoprotein cholesterol (HDL-C) was increased. Significant differences were observed between the sea-buckthorn high-dose treatment group and the high-fat model group \((P < 0.05)\). The extracts of sea buckthorn had a certain protective effect on non-alcoholic fatty liver. This study lays an important foundation in developing and using sea buckthorn extract as a clinical drug and guiding people to take health care products reasonably.

Keywords: Hipphophae rhamnoides; High fat diet; Fatty liver; Hypolipidemic Effect

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is caused by factors other than alcohol and specific liver injury, mainly characterized by excessive fat deposition in hepatocyte\(^1\). Changes in diet and lifestyle have led to the continued prevalence of metabolic related

---

\(^1\) Corresponding author: Lian HE, College of Basic Medicine, Changsha Medical University, Changsha, Hunan 410219, China; helian4260@sina.com
diseases. NAFLD has developed into a global health problem that poses a threat to human health, affecting approximately 24% of the global population. Treatment for NAFLD includes non-drug therapy (lifestyle change) and drug therapy. Lifestyle change is the basis of treatment for NAFLD, and drug treatment mainly includes insulin sensitizer, lipid-lowering drugs, liver protection anti-inflammatory drugs, etc. Many studies have been conducted on NAFLD therapeutic drugs. At present, clinically used drugs for the treatment of fatty liver injury include pioglitazone and liraglutide. Lewis et al. [2] found that pioglitazone can improve steatosis, lobular inflammation, hepatocyte balloon-like degeneration, and total NAFLD activity score, but the safety of pioglitazone remains to be discussed. Pioglitazone can lead to increased blood volume and mild-to-moderate edema in 4.8% of patients, and it can even cause or worsen congestive heart failure. Armstrong et al. [3] found that liraglutide can be used to treat type 2 diabetes by promoting glucose-concentration-dependent insulin secretion. Liraglutide (1.8 mg/day, subcutaneously injected) treatment for 1 year could improve steatohepatitis and prevent fibrosis progression. However, liraglutide requires subcutaneous injection, and gastrointestinal side effects are common [3–4]. Therefore, developing new, efficient, and low-side-effect drugs to protect the liver has become a hot topic in modern pharmacology.

Sea buckthorn (Hipphophae rhamnoides) primarily distributed in north, northwest, and southwest China. Sea buckthorn fruit is rich in isorhamnosin, quercetin, kaempferol, myricetin, rutin, catechin, flavonoids and other substances, which indicates its high medicinal value. According to modern pharmacological studies, the active components of sea buckthorn have various biological functions, such as promoting blood circulation and lowering blood lipids; it also shows antioxidant, anti-atherosclerosis, hypoglycemic, anti-tumor, and anti-inflammatory effects [5–7]. Compared with western medicines, the toxic side effects of sea buckthorn are smaller, and its cost is lower. Therefore, this paper aims to investigate the protective effect of natural sea buckthorn juice on NAFLD mice established by high-fat and high-cholesterol diet.

2. Materials and methods

2.1 Materials and methods

SPF-grade male KM mice, weighing approximately 20 g, were purchased from Changsha Tianqin Biological Company. The experiment was carried out after 7 days of adaptive feeding in the animal house. The mice ate and drank freely, under natural light and at room temperature of 22 ℃–25 ℃.

All kits of detection of serological related biochemical indicators were purchased from Nanjing Jiancheng Bioengineering Institute.

2.2 Experimental materials

High-fat and high-cholesterol diet: 0.2% bile salt, 20% lard, 2% cholesterol, 16% white sugar, and high-protein nutrition basic diet (lot number: 202133, purchased from Shengmin Scientific Research Animal Farm, Nanjing). In the laboratory, fresh fruits of sea buckthorn were squeezed using gauze to extract and filter sea buckthorn juice.
2.3 Experimental methods

2.3.1 Experimental grouping
The mice were randomly divided into five groups, with five mice in each group. The high-fat model group and sea-buckthorn treatment group were fed high-fat and high-cholesterol diet, whereas the blank control group was fed ordinary diet. The sea-buckthorn juice low-dose, medium-dose, and high-dose groups were intragastrically given 5, 10, and 20 mL/kg of sea buckthorn juice, respectively, whereas the blank control group and high-fat model group were intragastrically given saline solution. The amount of sea buckthorn juice was adjusted weekly in accordance with the weight of mice. From the first day of the experiment, all mice were continuously treated according to the abovementioned method for 35 days.

2.3.2 Determination of relevant serological indexes
The blood collected from the mouse subocular vein was placed into a centrifuge tube and left to stand. The blood sample was centrifuged to separate the serum. The content of TC, TG, HDL-C, LDL-C, ALT, and AST was determined by microplate reader according to the method in the kit.

2.3.3 Calculation of liver coefficient
The mice in each group were euthanized. After weighing the mice, they were dissected, and their livers were weighed. The liver coefficient was calculated in accordance with the following formula:

\[ \text{liver coefficient (\%)} = \frac{\text{liver weight}}{\text{mouse weight}} \times 100\% \]

2.3.4 Data analysis
All data were expressed as \( \bar{x} \pm s \), and SPSS 20.0 was used for statistical processing. Differences among the groups were tested by one-way analysis of variance. \( P < 0.05 \) indicated a statistically significant difference between groups.

3. Results

3.1 Effects of sea buckthorn juice on blood lipid contents of NAFLD mice
Blood Lipids are the general name of lipid substances in plasma, which not only supply and store energy for the body, but also maintain the normal structure and function of biofilm. Dyslipidemia and hepatocyte steatosis are closely related to the pathological process of NAFLD. Compared with the blank control group, the content of TC and TG in serum of mice in the high-fat model group was significantly increased \( (P < 0.05) \), indicating that the high-fat mouse model was successfully established (Table 1). The content of TC, TG and LDL-C in serum of sea buckthorn juice treated mice were decreased to varying degrees, while the content of HDL-C was increased, and the high lipid level of NAFLD mice was improved (Table 1). Specifically, the content of TG in serum of high-dose group mice was extremely significantly decreased \( (P < 0.01) \), the content of TC and LDL-C was significantly decreased \( (P < 0.05) \), and the content of
HDL-C was significantly increased ($P < 0.05$). Our results suggested sea buckthorn juice can significantly improve the serological indexes of NAFLD mice, with high-dose sea buckthorn juice having the best lipid-lowering effect.

### Table 1. Changes of blood lipids of mice

<table>
<thead>
<tr>
<th>group</th>
<th>TC (mmol/L)</th>
<th>TG (mmol/L)</th>
<th>HDL-C (mmol/L)</th>
<th>LDL-C (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank control group</td>
<td>1.99±0.31**</td>
<td>0.99±0.21*</td>
<td>3.76±0.30**</td>
<td>0.99±0.11**</td>
</tr>
<tr>
<td>High-fat model group</td>
<td>3.77±0.38</td>
<td>1.55±0.45</td>
<td>2.92±0.24</td>
<td>2.05±0.20</td>
</tr>
<tr>
<td>Low-dose group</td>
<td>3.63±0.19</td>
<td>0.99±0.27*</td>
<td>3.26±0.29</td>
<td>1.87±0.23</td>
</tr>
<tr>
<td>Medium-dose group</td>
<td>3.46±0.39</td>
<td>0.98±0.15*</td>
<td>3.51±0.51</td>
<td>1.79±0.17</td>
</tr>
<tr>
<td>High-dose group</td>
<td>3.16±0.76*</td>
<td>0.66±0.12**</td>
<td>3.64±0.33*</td>
<td>1.64±0.40*</td>
</tr>
</tbody>
</table>

Note: Comparison with high-fat model group, *: $P<0.05$; **: $P<0.01$. These two symbols have the same meaning in all the tables below, so the description is not repeated.

### 3.2 Effects of sea buckthorn juice on serum AST and ALT contents of mice

In chronic hepatobiliary diseases, the activity of ALT and AST in blood is an important index necessary for liver function experiments. When hepatocytes are injured or necrotic, transaminase metabolism is abnormal, leading to the elevation of ALT and AST levels. Compared with the high-fat model group, the content of ALT and AST in serum of mice in all sea-buckthorn-treated groups decreased with the increase of the intragastric dose of sea buckthorn juice, and the content of AST and ALT in serum of high-dose group mice could significantly decrease ($P < 0.05$) (Table 2).

### Table 2. Changes of ALT, AST in serum of mice

<table>
<thead>
<tr>
<th>group</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-fat model group</td>
<td>57.13±16.90</td>
<td>127.37±19.56</td>
</tr>
<tr>
<td>Low-dose group</td>
<td>43.32±8.84</td>
<td>106.00±8.64</td>
</tr>
<tr>
<td>Medium-dose group</td>
<td>38.63±7.64*</td>
<td>95.97±15.00</td>
</tr>
<tr>
<td>High-dose group</td>
<td>35.88±8.28*</td>
<td>84.33±22.11*</td>
</tr>
</tbody>
</table>

### 3.3 Effect of sea buckthorn juice on the liver coefficient of mice

When the liver coefficient increases, the liver is likely to develop edema, congestion, or hyperplasia and hypertrophy. Otherwise, the liver may undergo atrophy or other degenerative changes. The proportion of the liver in high-fat diet mice was larger than that in the sea buckthorn treatment group and the proportion of the liver gradually decreased with the increase of sea buckthorn dose, showing an evident dose dependence (Table 3). The liver coefficient of mice was extremely significantly decreased by high-dose of sea buckthorn juice ($P < 0.01$).
Table 3. Effect Changes of liver coefficient of mice

<table>
<thead>
<tr>
<th>group</th>
<th>liver coefficient (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-fat model group</td>
<td>5.39±0.52</td>
</tr>
<tr>
<td>Low-dose group</td>
<td>4.95±0.59</td>
</tr>
<tr>
<td>Medium-dose group</td>
<td>4.53±0.64*</td>
</tr>
<tr>
<td>High-dose group</td>
<td>4.10±0.33**</td>
</tr>
</tbody>
</table>

4. Discussion

Hyperlipidemia refers to the high level of blood lipids, which can cause some diseases, such as atherosclerosis and pancreatitis [9]. High-fat nutritious foods, after digestion and absorption, form free fatty acids and then circulate in the liver to form TG, which can enter the blood as very low density lipoprotein (VLDL) or be stored in lipid drops. High-fat diet mice are accompanied by changes of biochemical indicators in serum. The mechanism may be that when fatty acids are supplied in excess, TG can be used as the substrate produced by lipotoxic substances to cause liver cell damage, leading to oxidation of free fatty acids and reduction of VLDL, reducing the transport of TG out of liver cells, resulting in the deposition of TG in hepatocyte, and finally inducing NAFLD [9]. Therefore, serum lipid levels can be used as the pivotal criterion for evaluating the progression of NAFLD.

Sea buckthorn is a kind of medicinal and edible plant, which is rich in a variety of chemical components, including flavonoids, vitamins, carotenoids, sterols, volatile oils and so on. Flavonoids, the main active component of sea buckthorn, have good effects in the treatment of heart disease, hypertension, hyperlipidemia, hyperglycemia and tumor [6-7]. Wang et al. [5] found that flavonoids extract from sea buckthorn seeds can reduce the body weight and blood lipid of high-fat model mice, as well as the contents of TC and LDL-C, suggesting that the total flavonoid extract from sea buckthorn seeds has the effect of lowering blood lipids and reducing body weight. In this experiment, sea buckthorn juice can significantly reduce the content of TG, TC, and LDL-C in the serum of mice and increase the content of HDL-C. Reduction in the content of TG is the most evident effect, which directly reflects the content of free fatty acids in the serum of mice, suggesting that the extract of sea buckthorn can delay the pathological process of NAFLD by reducing the free fatty acids in blood and improving hyperlipidemic in mice. We speculated that the flavonoids of sea buckthorn juice played an important role in reducing blood lipids.

Liver is not only an important metabolic organ of the body, but also has important functions such as secretion, excretion and detoxification. High-fat diet causes excessive lipid substances to enter the liver, leading to the imbalance of lipid metabolism, lipid deposition, and hepatocyte necrosis. Hepatic steatosis is the signature feature of NAFLD, accompanied by elevated serum AST and ALT levels. In most cases, the elevation of AST and ALT are consistent with the extent of hepatocyte damage [10]. In this study, the content of liver function indexes in sea-buckthorn-treated mice were decreased, which reflected the weakened peroxidation in the liver of mice, indicating that sea buckthorn juice had a certain protective effect on mouse liver.
In this paper, the protective effect of sea buckthorn juice on liver damage was studied by inoculating animals with different concentrations of sea buckthorn juice, and related serological indexes were determined. Subsequent studies should clarify specific active components of sea buckthorn extract and the specific mechanism of active components in reducing the lipid contents of blood lipid and improving liver function to lay an important foundation for the development of sea buckthorn extract as a clinical drug.

5. Conclusions

In summary, we investigated the protective effect of sea buckthorn extract on NAFLD animal models. The results indicated that high-dose sea buckthorn juice can significantly improve blood lipid levels and liver function in NAFLD mice. This study provides an important experimental basis for promoting the use of sea buckthorn extract as a drug for the treatment of NALFD.

Experimental Ethics Statement

All animal experiments were approved by the Medical Ethics Committee of Changsha Medical University.

Acknowledgment

This work was supported by the National College Students Innovation and Entrepreneurship Training Program (S202010823003) and the Scientific Research Fund for the Talent Introduction of Changsha Medical University.

References