Antioxidant Activity of Aqueous Extract of Spatholobi Caulis and Its Ameliorative Effect on CCl4 Induced Acute Liver Injury in Mice

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Abstract: The acute liver injury model of mice induced by carbon tetrachloride (CCl4) was used to explore the protective effect of aqueous extract of Spatholobi Caulis on acute liver injury. Methods: The contents of total phenols and total flavonoids in the aqueous and ethanol extracts of Caulis spatholobi were determined, and the antioxidant activity was evaluated by DPPH, ABTS free radical scavenging rate and FRAP total reducing capacity. 30 C57BL/6J male mice were randomly divided into model group, 10 groups dose of aqueous extract of Spatholobi Caulis (n = 6). The animal model of acute liver injury was established by intraperitoneal injection of CCl4 after 7 days with corresponding drugs. The pathological changes of liver tissue were observed by H&E staining. Serum levels of interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-10 (IL-10) and tumor necrosis factor α (TNF-α) were determined by ELISA. The indexes of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and antioxidant effects of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and malondialdehyde (MDA) were determined. 

Results: Liver histomorphology observation showed that aqueous extract of Spatholobi Caulis could significantly improve liver cell injury and inflammatory cell infiltration, and significantly increased the activities of CAT, SOD and GSH-Px antioxidant enzymes in liver; decreased the contents of MDA and ALT and AST in serum, significantly down-regulated the levels of IL-1β, IL-6 and TNF-α in serum, and up-regulated the levels of IL-10 and other inflammatory factors. Conclusion: The aqueous extract of Spatholobi Caulis can improve acute liver injury induced by CCl4 in mice through improving antioxidant and anti-inflammatory effects.

Keywords: Spatholobi Caulis, Aqueous extract, Acute liver injury, CCl4

1. Introduction

The liver is the largest internal organ and plays an important role in the metabolism of substances in the human body. Liver injury causes serious harm to the body and it is an important factor leading to liver fibrosis, liver failure, cirrhosis, and liver cancer [1]. In recent years, the morbidity and mortality of acute liver injury have been on the rise [2]. Acute liver in-jury refers to abnormal liver function caused by sudden liver cell damage caused by various reasons in a short period of time, among which acute liver injury caused by drug action is the main cause, and its incidence is increasing year by year [3]. So far, there are no effective preventive and therapeutic measures for acute liver injury clinically. Therefore, the development of therapeutic drugs for acute liver injury is of great significance.

Traditional Chinese medicine has attracted much attention in the prevention and treatment of liver diseases because of its good clinical efficacy and small side effects. A variety of traditional Chinese medicines have been proven to protect the liver [4,5], and have been widely used in clinical treatment. Spatholobi Caulis is the dried cane of the leguminous plant Spatholobi Caulis, which has the effects of nourishing blood, promoting blood circulation, and dredging collaterals [6]. Studies have reported that Spatholobi Caulis can inhibit oxidative stress, reduce mitochondrial damage, and has anti-tumor, anti-
inflammatory, anti-oxidation, and immune regulation effects\textsuperscript{[7,10]}. In addition, \textit{Spatholobi Caulis} also has a good effect on liver and kidney protection\textsuperscript{[11]}. Therefore, we speculate that \textit{Spatholobi Caulis} has a certain protective effect on liver inflammatory injury.

Carbon tetrachloride (CCl\textsubscript{4}) causes liver injury by stimulating liver cells, which is similar to the mechanism of acute liver injury in humans\textsuperscript{[12]}, so it is widely used to induce animal models of acute liver injury. During the establishment of the model, CCl\textsubscript{4} induces liver cells to secrete a large number of inflammatory factors mainly by inducing physiological and biochemical reactions such as inflammatory response and oxidative stress in the liver, thereby aggravating inflammation and causing liver damage\textsuperscript{[13,14]}. In the process of acute liver injury, due to the imbalance of oxidation and antioxidant system in the body, the oxidative stress response is activated, and a large number of oxidation products are produced to induce a series of inflammatory reactions and aggravate the disease progression\textsuperscript{[13]}. Therefore, this experimental study aims to use the aqueous extract of \textit{Spatholobi Caulis} (AES) to intervene in the acute liver injury model induced by CCl\textsubscript{4} in mice, and to explore the ameliorative effect of \textit{Spatholobi Caulis} on acute liver injury and its potential mechanism, and provide a traditional Chinese medicine treatment method for the clinical treatment of acute liver injury.

2. Material and methods

2.1 Experimental animals

Thirty SPF-grade C57BL/6J male mice, body weight 20-24 g, were purchased from Hunan Slake Jingda Experimental Animal Co., Ltd, license number: SCXK (Xiang) 2019-0004.

2.2 Main reagents

\textit{Spatholobi Caulis} (identified and kindly provided by researcher Kunhua Wei of Guangxi Medicinal Botanical Garden), bifendate (Shanghai McLean Biochemical Technology Co., Ltd.), ALT, AST, CAT, SOD, GSH-Px and MDA determination kit (Nanjing Jiancheng Bioengineering Institute), IL-1β, IL-6, IL-10 and TNF-α ELISA kit (Quanzhou Ruixin Biotechnology Co., Ltd.).

2.3 Main instruments and equipment

Avanti JXN-26 high-speed refrigerated centrifuge (Shanghai Yubo Biotechnology Co., Ltd.), JY88-11N ultrasonic cell disruptor (Tuohe Electromechanical Technology Shanghai Co., Ltd.), UV-2700 ultraviolet spectrophotometer (Shimadzu Instrument Co., Ltd., Japan), VarioskanLuX microplate reader (ThermoFisher, USA).

2.4 Determination of Total phenols and total flavonoids content and antioxidant activity of different extracts of Spatholobi Caulis

The determination of total phenols content refers to the method of Pengkumsri et al\textsuperscript{[16]}, the content is expressed in gallic acid equivalent (GAE), the unit is mg/g extract, the standard curve of gallic acid 
\[ Y=0.979X+0.1509, R^2=0.9906. \]

The content of total flavonoids was determined according to the method of Moreno et al\textsuperscript{[17]}, and the content was expressed in rutin equivalent (RE). The standard curve of rutin 
\[ Y=1.5353X+0.0512, R^2=0.9996. \]

ABTS free radical scavenging activity was measured according to the method of Re et al\textsuperscript{[18]}, DPPH free radical scavenging activity was measured according to the method of Blois et al\textsuperscript{[19]}, and FRAP total antioxidant capacity was measured according to the method of Benzie et al\textsuperscript{[20]}. The antioxidant activity of different extracts of \textit{Spatholobi Caulis} was evaluated by ABTS, DPPH free radical scavenging activity and FRAP total reducing ability, and an ideal extract of \textit{Spatholobi Caulis} was selected to intervene in mice.

2.5 Animal Grouping and Handling

After 7 days of normal feeding, 30 C57BL/6J male mice were randomly divided into CCl\textsubscript{4} liver injury model (Model) group, drug bifendate (Bifendate) group (3.75 mg·kg\textsuperscript{-1}·d\textsuperscript{-1}), the low-dose (AES-L) group (50 mg·kg\textsuperscript{-1}·d\textsuperscript{-1}), medium-dose (AES-M) group (100 mg·kg\textsuperscript{-1}·d\textsuperscript{-1}) and high-dose (AES-H) group (150 mg·kg\textsuperscript{-1}·d\textsuperscript{-1}) of aqueous extract of \textit{Spatholobi Caulis}. The mice in the Model group were given a placebo of 0.01 mL·g\textsuperscript{-1} carboxymethylcellulose sodium (CMC-Na) solution every day, and the mice in the other
groups were intragastrically administered with the corresponding drug in an equal volume mixture for 7 consecutive days. The drug was suspended and mixed evenly in 0.01 mL·g⁻¹ carboxymethylcellulose sodium (CMC-Na), and 2 hours after the end of the last gavage, all mice were intraperitoneally injected with 0.01 mL·g⁻¹ CCl₄ to induce acute liver injury damage.

2.6 Liver index determination and Histopathological observation of mouse liver

Calculate liver coefficient formula: Liver index (%) = (liver weight/body weight) × 100%. The liver tissue fixed in 4% paraformaldehyde solution was embedded in paraffin and sectioned, and the histopathological changes of mouse liver were observed after H&E staining.

2.7 Antioxidant enzyme activity assay and measurement of blood indicators

The liver tissue was weighed, chopped and placed in a homogenize, and an appropriate amount of 4°C pre-cooled normal saline was added to prepare 10% liver tissue homogenate, which was then centrifuged at 3000 rpm/min for 10 min in a 4 °C centrifuge. The supernatant was taken to determine the activities of CAT, SOD, GSH-Px and the content of MDA in liver tissue according to the instructions of the kit. Serum ALT, AST, IL-1β, IL-6, IL-10 and TNF-α were measured according to the instructions of the kit.

2.8 Statistical methods

Statistical analysis was performed using GraphPad Prism 8.0.1 statistical software (GraphPad Software Inc., San Diego, CA, United States). All experimental data were expressed as mean ± standard deviation. The Student’s t-test was used to compare the means between two datasets, and one-way analysis of variance (ANOVA) was used to compare the means between three or more data points. Differences with p-values less than 0.05 were considered statistically significant.

3. Results

3.1 Determination results of total phenols and total flavonoids in different extracts of Spatholobi Caulis

The contents of total phenols and flavonoids in the aqueous extract were higher than those in the ethanol extract, and the content of total flavonoids was significantly different (P<0.05). The higher content of total phenols and total flavonoids in the aqueous extract of Spatholobi Caulis indicates that Spatholobi Caulis has stronger solubility in water and more adequate extraction. (Table 1)

<table>
<thead>
<tr>
<th>Group</th>
<th>Total phenols (mg/g extract)</th>
<th>Total flavonoids (mg/g extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous extract of Spatholobi Caulis</td>
<td>0.95±0.02</td>
<td>0.33±0.02*</td>
</tr>
<tr>
<td>Ethanol extract of Spatholobi Caulis</td>
<td>0.91±0.01</td>
<td>0.28±0.03</td>
</tr>
</tbody>
</table>

Notes: *P<0.05 compared with ethanol extract of Spatholobi Caulis.

3.2 Determination results of antioxidant activity of different extracts of Spatholobi Caulis

As shown in Figure 1A and Figure 1B, with the increase of different extracts concentration, the scavenging rates of ABTS and DPPH free radicals were significantly increased, and at higher concentrations, the ABTS and DPPH free radical scavenging abilities of aqueous extracts were stronger than ethanol extracts of Spatholobi Caulis. The results of the total reducing power of FRAP were shown in Figure 1C, within a certain concentration range, the total reducing power of FRAP of aqueous extract of Spatholobi Caulis and ethanol extract was positively dependent on the concentration, and the total reduction capacity of FRAP in the aqueous extract of Spatholobi Caulis is better than that in the ethanol extract of Spatholobi Caulis. The results of this study showed that compared with the ethanol extract of Spatholobi Caulis, the aqueous extract of Spatholobi Caulis has better antioxidant capacity. (Figure 1)
3.3 Effects of aqueous extract of Spatholobi Caulis on liver weight and liver index in mice with acute liver injury

Liver weight and liver index are important indexes to evaluate liver injury. The results showed that the Model group of mice had the highest body weight, liver weight and liver index. Compared with Model group, liver weight in Bifendate group and AES-M group was significantly decreased (P<0.05). After the intervention of aqueous extract of Spatholobi Caulis, the liver indexes of AES-L and AES-M mice were decreased (P<0.05), and the indexes of AES-M group were closer to Bifendate group. (Table 2)

Table 2: Effects of aqueous extract of Spatholobi Caulis on liver weight and liver index in mice with acute liver injury (x±s, n = 6)

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (g)</th>
<th>Liver weight (g)</th>
<th>Liver index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>23.49±1.12</td>
<td>1.33±0.10</td>
<td>5.66±0.29</td>
</tr>
<tr>
<td>Bifendate</td>
<td>22.50±1.29</td>
<td>1.05±0.12</td>
<td>4.65±0.29***</td>
</tr>
<tr>
<td>AES-L</td>
<td>22.82±1.96</td>
<td>1.13±0.14</td>
<td>4.94±0.25**</td>
</tr>
<tr>
<td>AES-M</td>
<td>22.15±1.38</td>
<td>1.07±0.15</td>
<td>4.80±0.49**</td>
</tr>
<tr>
<td>AES-H</td>
<td>22.29±1.59</td>
<td>1.13±0.17</td>
<td>5.07±0.46</td>
</tr>
</tbody>
</table>

Notes: *P<0.05, **P<0.01, ***P<0.001 compare with Model group.

3.4 Effect of aqueous extract of Spatholobi Caulis on pathological morphologic changes of liver tissue in mice

The results showed that large areas of liver tissue in the Model group were blurred and necrotic, and some of the liver tissues were accompanied by inflammatory cell infiltration. Compared with Model group, Bifendate group and AES-L, AES-M and AES-H groups could reduce liver tissue damage to different degrees. The results showed that the aqueous extract of Spatholobi Caulis could improve acute liver injury induced by CCl₄ in mice. (Figure 2)

Figure 2: Effects of aqueous extract of Spatholobi Caulis on pathological changes of liver tissue in mice (H&E×100, H&E×200). The red arrow represents liver tissue necrosis, and the black arrows represent inflammatory cell infiltration.

3.5 Effects of aqueous extract of Spatholobi Caulis on serum biochemical indices of mice

ALT and AST are important transaminases in the body and are commonly used in the detection of liver function indicators. Elevated ALT reflects liver cell membrane damage, and elevated AST indicates liver organelle damage [21]. Hepatocyte injury will lead to a significant increase in serum ALT and AST indicators [22]. As shown in Figure 3A and Figure 3B, the release of ALT and AST enzyme activities in
serum of mice in the Model group was the highest in each group of mice, indicating that CCl₄ caused serious damage to the liver tissue of the mice in the Model group. Compared with the Model group, the levels of ALT and AST in the serum of the mice in the Bifendate group and the AES-L, AES-M, and AES-H groups were significantly lower (P<0.001). The results indicated that the intervention with aqueous extract of Spatholobi Caulis could significantly relieve the elevated serum ALT and AST levels caused by CCl₄ in mice. Compared with the mice in the Bifendate group, the levels of ALT and AST in the serum of the mice in the AES-L group were significantly increased (P<0.01). (Figure 3)

Figure 3: Effect of aqueous extract of Spatholobi Caulis on serum ALT and AST contents in mice (x±s, n = 6), ***P<0.001 compare with Model group, and ΔP<0.05, ΔΔP<0.01, ΔΔΔP<0.001 compare with Bifendate group.

3.6 Effect of aqueous extract of Spatholobi Caulis on serum inflammatory factors in mice

IL-1β, IL-6 and TNF-α are important inflammatory factors in the body. After the body has an inflammatory response, the level of inflammation in the body will increase significantly [23, 24]. The results in Table 3 showed that the serum levels of inflammatory cytokines in the Model group were significantly increased. Compared with the Model group, the levels of IL-1β, IL-6 and TNF-α in the serum of the mice in the drug intervention group were significantly reduced (P<0.05). In addition, IL-10 as an important regulator of inflammation can reduce the levels of pro-inflammatory cytokines such as IL-1β, IL-6 and TNF-α, thereby reducing inflammatory damage [25]. Compared with the Model group, the level of IL-10 in the Bifendate group was significantly increased (p<0.001), and the level of IL-10 in the AES-M group was also significantly different (P<0.05). The results showed that Spatholobi Caulis can reduce the inflammatory response of mouse liver tissue caused by CCl₄ through its anti-inflammatory effect. (Table 3)

Table 3: Effects of aqueous extract of Spatholobi Caulis on serum inflammatory factors in mice with acute liver injury (x±s, n = 6, pg/mL serum)

<table>
<thead>
<tr>
<th>Group</th>
<th>IL-1β</th>
<th>IL-6</th>
<th>TNF-α</th>
<th>IL-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>39.3±5.35</td>
<td>51.5±7.97</td>
<td>206.5±20.35</td>
<td>126.8±25.00</td>
</tr>
<tr>
<td>Bifendate</td>
<td>19.7±5.55</td>
<td>34.5±5.00</td>
<td>139.3±17.64</td>
<td>204.5±18.82</td>
</tr>
<tr>
<td>AES-L</td>
<td>29.6±5.57</td>
<td>43.1±5.53</td>
<td>172.6±15.16</td>
<td>153.1±20.47</td>
</tr>
<tr>
<td>AES-M</td>
<td>24.3±3.62</td>
<td>39.3±5.35</td>
<td>157.8±8.77</td>
<td>167.5±21.38</td>
</tr>
<tr>
<td>AES-H</td>
<td>34.6±6.26</td>
<td>34.6±6.26</td>
<td>141.0±13.17</td>
<td>131.6±22.85</td>
</tr>
</tbody>
</table>

Notes: *P<0.05, **P<0.01, ***P<0.001 compare with Model group, and ΔP<0.05, ΔΔP<0.01, ΔΔΔP<0.001 compare with Bifendate group. The same as the following table

3.7 Effects of aqueous extract of Spatholobi Caulis on oxidative stress indexes in liver tissue of mice with acute liver injury

Oxidative stress is the common pathophysiological basis of various liver injuries. CAT, SOD, GSH-Px and MDA can reflect the degree of lipid peroxidation and oxidative stress [26]. Compared with Model group, the contents of CAT, SOD and GSH-Px in the liver tissue of mice in the intervention group were significantly increased, while MDA levels in different dosage groups of Aqueous extract of Spatholobi Caulis were decreased (P<0.05). Compared with Bifendate group, SOD activity in AES-L and AES-H groups was significantly decreased (P<0.001). The results showed that CCl₄ could cause the imbalance of oxidation and antioxidant, and the AES-M group could significantly up-regulate the activities of CAT, SOD and GSH-Px, and reduce the level of MDA to relieve oxidative stress, so as to improve the liver injury induced by CCl₄ in mice. (Table 4)

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4. Discussion

As the largest parenchymal metabolic organ in the human body, the liver’s functions include metabolism and decomposition of substances, removal of toxins, and anti-oxidation. Because of its good effect and high modeling rate, CCl₄ has been widely used to induce animal models of acute liver injury. Studies have confirmed that intraperitoneal injection of CCl₄ into the animal body can be quickly absorbed by the liver, and under the action of intrahepatic cytochrome P450, toxic free radicals such as trichloromethyl free radicals and chlorine free radicals are generated, causing cell membrane and organelle membrane lipid hyperactivity. Oxidative reactions, resulting in structural and functional disturbances of cell membranes, eventually causing acute severe injury and necrosis of hepatocytes \([27,28]\).

ALT and AST are important indicators of liver damage. After liver cell injury, the levels of ALT and AST in serum increased significantly \([29]\). This study found that the serum ALT and AST levels of mice in the Model group were significantly higher than those in the drug intervention group. Combined with the results of mouse liver index and H&E staining, administration of different doses of \textit{Spatholobi Caulis} extract by gavage can reduce AST and ALT indexes, reduce liver index to varying degrees, and significantly improve the state of liver damage. In addition, we also found that the effect of medium and high doses of aqueous extract of \textit{Spatholobi Caulis} on acute liver injury is comparable to that of bifendate. This suggests that the aqueous extract of \textit{Spatholobi Caulis} can be used as a potential drug to replace bifendate.

Oxidative stress is one of the important mechanisms of CCl₄ induced liver cell injury and necrosis \([30]\). CAT, SOD and GSH-Px, as important antioxidant enzymes in the body, are sensitive indicators reflecting oxidative stress \([21]\). Liver tissue damage during oxidative stress leads to an increase in reactive oxygen species (ROS), and antioxidant enzymes such as CAT, SOD, and GSH-Px in the body can effectively prevent this damage process \([31]\). In this study, we found that the activities of SOD, GSH-Px and CAT in the liver of mice treated with CCl₄ were significantly down-regulated, and the aqueous extract of \textit{Spatholobi Caulis} significantly increased the activities of SOD, GSH-Px and CAT in the liver tissue of mice and the effect of the middle-dose group was equivalent to that of the drug bifendate group, indicating that an appropriate amount of aqueous extract of \textit{Spatholobi Caulis} has the antioxidant function of reversing the acute liver injury caused by CCl₄ in mice. In addition, it has been reported that the production of MDA is closely related to the pathological mechanism of liver toxicity, and elevated levels of MDA in the liver lead to aggravated liver tissue damage and decreased antioxidant function \([32,33]\). The experimental results show that the aqueous extract of \textit{Spatholobi Caulis} can inhibit the increase of MDA content in mice induced by CCl₄, which may be due to the strong antioxidant and free radical scavenging ability of \textit{Spatholobi Caulis}. Thus, reducing the oxidative damage caused by CCl₄ to the liver.

Inflammation is an important factor that exacerbates liver damage. Studies have reported that in the process of CCl₄ induced liver cell injury, oxidative stress can activate inflammatory cells, causing them to release a large number of pro-inflammatory factors to further induce inflammatory responses \([34]\). The increase in the levels of pro-inflammatory cytokines IL-1β, IL-6 and TNF-α in the Model group of mice in this study may lead to the accumulation of immune cells, thereby inducing their apoptosis and eventually leading to liver injury. TNF-α is a key inflammatory cytokine released in the early stage of liver cell damage, which can activate neutrophils to release proteases and oxygen free radicals, leading to apoptosis or necrosis of liver cells \([35]\). IL-6 is a cytokine closely related to inflammation. It participates in immune response by activating and regulating immune cells. IL-1β can not only cause severe inflammatory response, but also induce the expression of inflammatory factors and participate in acute inflammation \([36]\). IL-10 is an important regulator of inflammation, which can inhibit the expression of pro-inflammatory factors such as IL-1β, IL-6 and TNF-α, thereby reducing the inflammatory injury of liver tissue \([37]\). The experimental results show that the use of medium and high doses of aqueous extract...
of Spatholobi Caulis can more effectively reduce the levels of inflammatory factors IL-1β, IL-6 and TNF-α in mice with liver injury. The extract can significantly increase the activity of IL-10, thereby inhibiting the inflammatory response.

In conclusion, the aqueous extract of Spatholobi Caulis has potential therapeutic effect on CCl₄ induced acute liver injury, and its target and pathway may be related to regulation of oxidative stress and inflammatory response. This study provides a theoretical basis for the clinical treatment of acute liver injury by Spatholobi Caulis in traditional Chinese medicine. Its related mechanism needs to be further studied.

Acknowledgments

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References


