Protective Effect of Ethanol Extract from Sweet Potato Leaves on CCL_4 -Induced Liver Injury in Mice

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Abstract [**Objectives**] To investigate the protective effect of ethanol extract from sweet potato leaves on liver injury induced by CCl_4 in mice. [**Methods**] 25 ICR mice were randomly divided into blank group, model group, high-dose extract group (200 mg/kg), low-dose extract group (100 mg/kg) and positive control group (2 mg/kg colchicine), with 5 mice in each group. All groups except the blank group were given intraperitoneal injection of 20% CCl_4 olive oil solution (2 mL/kg), and the blank group was given the same dose of olive oil solution three times a week. After 4 weeks, each administration group was given the corresponding dose of drugs (10 mL/kg), and the blank group and model group were given the corresponding amount of normal saline for 2 weeks. After the last intragastric administration, fasting was required, but water was allowed, blood was taken from eyeballs, and upper serum was taken by static centrifugation. Serum AST, ALT, CRP, IL-6 and SOD levels were detected by the kit. [**Results**] Compared with the blank group, the serum AST and ALT levels in the model group were significantly increased; compared with the model group, the ethanol extract of sweet potato leaves could decrease the levels of ALT, AST, CRP, IL-6 and increase the level of SOD in serum. [**Conclusions**] The ethanol extract of sweet potato leaves had protective effect on the mice with liver injury induced by CCl_4, and its mechanism may be to protect the liver by lowering enzymes, inhibiting inflammation and antioxidant stress.

Key words Sweet potato leaves, Ethanol extract, Liver injury, Protective effect

1 Introduction

Sweet potato leaves, also known as Digua leaves, refer to the tender leaves at the top of stems during the growth of sweet potatoes, and are the by-products often neglected during the planting of sweet potatoes. It is found that sweet potato leaves are rich in flavonoids, polysaccharides, polyphenols, proteins, vitamins and minerals, and have very high nutritional value[1]. Its extract has anti-tumor effect, anti-oxidation effect, anti-cardiovascular and cerebrovascular disease effect, anti-inflammatory effect and analgesic effect, immune regulation effect, hypoglycemic effect, antibacterial effect and antiviral effect, *etc.*[2] However, the development and utilization of sweet potato leaves in most areas of China is very limited. Most sweet potato leaves are used as animal feed or abandoned directly in farmland, and only a small part of them are put on the table. The yield of sweet potato in China accounts for 80% of the global total, while the utilization rate of sweet potato leaves is less than 10%[3], which causes a huge waste of resources. The protective effect of ethanol extract from sweet potato leaves on liver injury induced by CCl_4 in mice was studied in order to provide a reference for further development and utilization of sweet potato leaves.

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2 Materials

2.1 Instruments FA12048 electronic balance (Shanghai Tianmei Balance Instrument Co., Ltd.); JSB-15 electronic weighing scale (Shanghai Puchun Measuring Instrument Co., Ltd.); L-530 low-speed centrifuge (Hunan Xiangyi Laboratory Instrument Development Co., Ltd.); HH-ZK600 intelligent thermostat water bath (Gongyi Yingyu Hi-Tech Instrument Factory); HHS-11-4 digital electrothermal thermostat water bath (Shanghai Baidian Instrument Equipment Co., Ltd.); RE-3000A rotary evaporator (Shanghai Yarong Biochemical Instrument Factory); DLSB-5/20 cryogenic coolant circulating pump and AHB-III circulating water multi-purpose vacuum pump (Zhengzhou Greatwall Scientific Industrial and Trade Co., Ltd.); TriStar LB941 multifunctional microplate reader (Berthold, Germany).

2.2 Experimental drugs and reagents Sweet potato leaves (purchased from Dongfeng Vegetable Market in Baise City) were identified as leaves on the stems of *Ipomoea batatas* (L.) Lam. in the Convolvulaceae family by Professor Huang Suoyi from Youjiang Medical University for Nationalities; 95% ethanol (analytically pure, Chengdu Kelong Chemicals Co., Ltd., batch No.: 2019061302); carbon tetrachloride (analytically pure, Tianjin Fengchuan Chemical Reagent Technology Co., Ltd., batch No.: 090516); Oleo Bella extra virgin olive oil (Jiangsu Jinzhou Cereals, Oils and Foodstuffs Co., Ltd., batch No.: F20220906); physiological saline (Sichuan Kelun Pharmaceutical Co., Ltd., batch No.: N22102901);

colchicine tablets (Yunnan Plant Pharmaceutical Co., Ltd., batch No.:20211012); alanine aminotransferase (ALT) kit, aspartate aminotransferase (AST) kit and total superoxide dismutase (SOD) kit (Nanjing Jiancheng Institute of Bioengineering, batch No.: C009-2-1, C010-2-1, A001-3-2, respectively); mouse interleukin-6 (IL-6) enzyme-linked immunosorbent assay kit and C-reactive protein (CRP) enzyme-linked immunosorbent assay kit (Shanghai Yuanju Biotechnology Center, batch No.: YJ009936, YJ002366, respectively).

2.3 Experimental animals 25 ICR mice, female, 6 weeks old, weighing (40 ± 5) g. Mice, bedding and feed were purchased from Guangdong Weitong Lihua Experimental Animal Technology Co., Ltd. and the certificate number was SCXK (Guangdong) 2022-0063. The mice were raised adaptively for one week before the formal experiment, and they were free to eat and drink water.

3 Methods

3.1 Preparation of ethanol extract from sweet potato leaves After the impurities were removed from purchased fresh sweet potato leaves, they were dried until completely dry, collected and crushed, and set aside in a dryer. 200 g of sweet potato leaf powder was weighed accurately, and then soaked in water for 24 h. The next day, it was heated with 2 000 mL of 60% ethanol for 2 h, the ethanol mixture was poured out and heated with 1 500 mL of ethanol for another 2 h. The ethanol mixture was poured out and the filtrate was combined twice. The filtrate was extracted to an ethanol-free state by using a rotary evaporator, and then the remaining liquid medicine was poured into a beaker and evaporated into a paste in an electric thermostatic water bath to obtain the ethanol extract of sweet potato leaves.

3.2 Grouping and administration 25 ICR mice were randomly divided into blank group, model group, high-dose extract group (200 mg/kg), low-dose extract group (100 mg/kg) and positive control group (2 mg/kg colchicine). There were 5 mice in each group, and the related solutions were allocated, respectively. All groups except the blank group were given intraperitoneal injection of 20% CCl_4 olive oil solution (2 mL/kg), and the blank group was given the same dose of olive oil solution, 3 times a week. After 4 weeks, each administration group was given the corresponding dose of drugs (10 mL/kg), and the blank group and model group were given the corresponding amount of normal saline for 2 weeks. After the last intragastric administration, fasting was required, but water was allowed, blood was taken from eyeballs, and the upper serum was obtained by 3 000 r/min centrifugation after resting for 30 min. Serum AST, ALT, CRP, IL-6 and SOD levels were detected by the kit.

3.3 Statistical methods SPSS 21.0 statistical analysis software was used to analyze and process the experimental data, and the results were expressed as $\bar{x} \pm s$. When $P < 0.05$, the difference was considered to be statistically significant; when $P > 0.05$, the difference was not statistically significant.

4 Results

4.1 General situation In the process of modeling, the mice in the blank group were generally in good condition, with normal mobility and smooth fur; the mice in the model group were generally in poor condition, their mobility was lower than that in the blank group, they were easy to lose hairs and their food intake decreased; the high-dose group, low-dose group and positive control group were better than the model group.

4.2 Effect of ethanol extract from sweet potato leaves on serum AST, ALT, CRP, IL-6, SOD in mice with liver injury Compared with the blank group, the serum levels of AST and ALT in the model group increased significantly ($P < 0.01$), indicating that the model of liver injury induced by CCl_4 was successfully established; compared with model group, high-dose group, low-dose group and positive control group can reduce serum AST and ALT levels. Compared with the blank group, the serum CRP and IL-6 levels in the model group increased significantly ($P < 0.01$). Compared with the model group, the serum CRP and IL-6 levels in the high-dose group, the low-dose group and the positive control group all decreased ($P < 0.01$, $P < 0.05$, respectively). Compared with the blank group, SOD activity in serum of model group decreased significantly ($P < 0.01$). Compared with model group, SOD activity in the serum of high-dose group, low-dose group and positive control group increased, as shown in Table 1.

Table 1 Effect of ethanol extract from sweet potato leaves on serum AST, ALT, CRP, IL-6, SOD in mice with liver injury ($n = 5$, $\bar{x} \pm s$)

Group	Dose//mg/kg	AST//U/L	ALT//U/L	CRP//pg/mL	IL-6//pg/mL	SOD//U/mL
Blank group	–	27.86 ± 3.66	9.28 ± 2.17	3.78 ± 0.26	12.10 ± 0.33	53.72 ± 3.46
Model group	–	41.28 ± 2.18$^{\#\#}$	24.40 ± 1.87$^{\#\#}$	5.68 ± 0.22$^{\#\#}$	16.57 ± 0.20$^{\#\#}$	34.75 ± 1.25$^{\#\#}$
Positive control group	2	31.85 ± 8.41*	10.92 ± 2.20**	4.75 ± 0.18**	12.07 ± 1.67*	45.25 ± 3.09**
High-dose group	200	28.63 ± 9.74**	10.08 ± 2.12**	3.76 ± 0.52**	12.84 ± 1.05*	50.93 ± 2.56**
Low-dose group	100	32.48 ± 5.56*	11.62 ± 4.01**	4.38 ± 0.36**	13.75 ± 0.95*	44.40 ± 1.60**

Note: – means no administration, $^{\#}$ means $P < 0.05$ when compared with blank group, $^{\#\#}$ means $P < 0.01$ when compared with blank group; * means $P < 0.05$ when compared with the model group, ** means $P < 0.01$ when compared with the model group.

5 Discussion

Liver, an important metabolic organ of human body, has many functions such as secretion and excretion of bile, detoxification and endocrine. If the liver is damaged, it will affect the normal metabolism and function of the body. The liver injury model induced by

CCl_4 (carbon tetrachloride) is a classical experimental liver injury model. Its mechanism is that after CCl_4 enters the body, a large number of free radicals are produced in liver cells and attack the biofilm structure, which leads to a large number of aspartate aminotransferases (ASTs) and alanine aminotransferases (ALTs) entering the blood, thus resulting in an increase in serum AST and ALT levels. Therefore, the increase of serum AST and ALT levels can reflect the injury of liver cells to a certain extent[4]. If liver injury persists, it may further develop into liver fibrosis or even liver cirrhosis. In this experiment, the serum levels of AST and ALT in the model group were significantly higher than those in the blank group ($P<0.01$), which indicated that the model of mouse liver injury induced by CCl_4 was successfully established. From the experimental results, compared with the model group, the high-dose and low-dose groups of ethanol extract from sweet potato leaves can reduce serum AST and ALT levels, which were close to those of the positive control group, and the effect of high-dose group was more significant ($P<0.01$), so sweet potato leaf can protect liver injury by protecting liver and reducing enzyme.

Modern studies have proved that inflammation is involved in the process of liver injury[4]. When inflammation occurs, mononuclear macrophages stimulate the liver by producing inflammatory factors such as IL-6, IL-1, and TNF-α, which increases the concentration of various proteins including CRP (C-reactive protein) in serum[5]. Therefore, CRP is often used as an important index to judge the changes of inflammation in the body clinically. This study confirmed that sweet potato leaves can reduce the levels of IL-6 and CRP in serum, and the effect of reducing CRP level was more significant. Therefore, sweet potato leaves can protect liver by inhibiting inflammatory reaction.

SOD, an important antioxidant enzyme in vivo, plays an important role in protecting the body. Studies have shown that the process of liver injury is accompanied by oxidative stress. After CCl_4 enters the body, it will stimulate the liver to undergo lipid peroxidation and produce a large quantity of peroxides to damage liver cells. In order to resist the damage caused by these substances, SOD will be produced in the liver to play an antioxidant role[6]. This experiment showed that sweet potato leaves can significantly increase the content of SOD in serum, and its effect was better than that of positive control group. The literature also confirmed that sweet potato leaves have strong antioxidant effect *in vitro*[7], so it can be considered that sweet potato leaves can protect liver through antioxidant stress.

To sum up, the ethanol extract of sweet potato leaves has a certain protective effect on the mice with CCl_4-induced liver injury, and its mechanism may be to protect liver by reducing enzyme, inhibiting inflammation and resisting oxidative stress. Liver injury is a common liver disease. If liver injury persists, it can cause liver fibrosis. At present, the intervention research on liver fibrosis mostly focuses on traditional Chinese medicine and its effective components[8], and there is less scientific research on food. Therefore, we can continue to study the effect of sweet potato leaves on liver fibrosis and further strengthen the development and utilization of sweet potato leaves.

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