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## Research Article

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## *Sedum emarginatum* and its polysaccharide have a therapeutic effect on alcoholic liver disease in mice

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### ABSTRACT

**Objective:** To explore the therapeutic effect of *Sedum emarginatum* (SE) and its polysaccharide on alcoholic liver disease. **Methods:** First, we ground fresh SE to obtain homogenate, and then test the effect of SE on a mouse model of acute alcoholic fatty liver disease (AFLD). Kunming female mice were given intragastric administration of alcohol once every 12 hours to induce AFLD, three times in total, and the mice received ethanol and SE at the same time. The mice were sacrificed 4 hours after the last alcohol administration, and serum and liver were collected for testing. We found that SE was effective and then carried out subsequent experiments. The dried SE powder was extracted under heating and reflux by different polar solvents: petroleum ether, ethyl acetate, methanol and water. The extracting solution were heat and concentrated to obtain an extract. The water part was purified to obtain polysaccharide. We tested the therapeutic effect of each part of SE in a mouse model of early alcoholic hepatitis (AH). Mice were given 5 g/kg of alcohol every 12 hours, a total of 9 times, and lipopolysaccharide (LPS) was injected intraperitoneally at the 36 h and 84 h. After 4 hours of the last gavage, the mice were sacrificed. The serum and liver tissue were used to tested relevant biochemical indexes. **Results:** SE had a certain reversal effect on the increase of serum triglycerides caused by alcohol, and it had a better effect on the accumulation of lipid droplets in liver tissues. Oil red O staining proved this result. In the second acute experiment, the mortality rate of the animal in the model group was 7/12, and the mortality rate in each treatment group was 1/8, 1/7, 4/8, and 1/8. In all parts, the crude polysaccharide did not significantly reverse the increase of serum triglyceride levels in early AH, but it could significantly alleviate the increase of inflammatory foci and steatosis in liver tissue. The transmission electron microscopy result indicates that the crude polysaccharide component has a certain protective effect on mitochondrial damage caused by alcohol. **Conclusion:** SE can reduce fatty liver and hyperlipidemia caused by alcohol, and its polysaccharide can reduce liver inflammation in the early alcoholic hepatitis by protecting mitochondrion.

**Keywords:** *Sedum emarginatum*, Alcoholic fatty liver, Alcoholic hepatitis, Polysaccharides.

### INTRODUCTION

According to a report by the World Health Organization in 2018, excessive alcohol consumption can cause 3.3 million deaths worldwide each year, accounting for 5.9% of all deaths [1]. The liver is the main part of ethanol metabolism in the human body and the main organ damaged by alcohol. Alcoholic liver disease (ALD) is a liver pathological change caused by long-term excessive drinking, including alcoholic fatty liver disease (AFLD), alcoholic hepatitis (AH), alcoholic hepatic fibrosis (AF) and alcoholic cirrhosis (AC) [2-4], etc. At present, in addition to liver transplantation, ALD is mainly treated through alcohol abstinence, nutritional supplements and drugs [5]. In this subject, we studied the *Sedum emarginatum* Migo (SE) of Crassulaceae. SE is rich in wild resources, often born in roadsides and stone crevices, and is widely used for medicinal consumption in Tujia areas of China. SE has the functions of clearing away heat and detoxification, cooling blood to stop bleeding, relieving pain and reducing swelling. In known experiments, SE mainly contains flavonoids such as isorhamnetin and quercetin, and its total flavonoids have been shown to be able to resist oxidation in vitro [6], sedative hypnosis [7], and have hemostatic and hypoxia-resistant effects [8], and it has a significant inhibitory effect on the proliferation of HepG2, EC109 and SW480 tumor cells [9]. At present, many active polysaccharides have been extracted from different medicinal plants [10]. Because of their different pharmacological activities, people have begun to pay attention. In the field of ALD, several animal and plant polysaccharides have been reported to have anti-AFLD effects, but there have been no reports of anti-AH. The high content of polysaccharides in SE has clinical application value and is worthy of further research and development. When surveyed in Badong County, Hubei Province, we found that SE was commonly used for the treatment of hepatitis by the native Tujia ethnic group, so this study will further explore the role of SE in treating alcoholic liver disease.

### MATERIALS AND METHODS

#### Collection, identification and preparation of the plant material

Fresh herbs of *Sedum emarginatum* were collected in Badong County, Hubei Province China (identified by Prof. Dingrong Wan, College of Pharmacy, South-Central University for Nationalities, Wuhan, China). The fresh SE was washed, dried, minced and placed in a mortar, and then ground with an appropriate amount of normal saline to obtain a homogenate so that it could pass through a gavage needle of a mouse with an outer diameter of 12 mm and a length of 4 cm for the first acute experiments. And other samples were dried and powdered for the second acute experiment.

### **Extraction procedure**

Dried powder (370 g) was extracted by heat reflux for 2 hours in 10 volumes of petroleum ether and filtered. The extraction was repeated three times. The filtrate was combined and concentrated to dryness. The filter residue was successively extracted in solvents of increasing polarity, including ethyl acetate, methanol, and water. The extraction of each solvents was repeated three times. The crude extracts were petroleum ether extract (PETE, 4.1 g), ethyl acetate extract (EACE, 12.2 g), methanol extract (ME, 52.0 g). In addition, 1.5% papain was added to the concentrated solution obtained from the aqueous extract, and the mixture was stirred and hydrolyzed at 60°C for 5 hours, then heated to 100°C, boiled for 10 minutes, and centrifuged to remove the precipitate. The precipitate obtained after alcohol precipitation of the resulting supernatant was washed once with anhydrous ethanol, acetone, and ether in order. The solvent was evaporated and lyophilized to obtain 30.6 g of crude polysaccharide, and the polysaccharide content was measured to be 63.8%. The extracts of each component were suspended in Erguotou at 10 mg/ml for further use.

### **Experimental animals**

Female Kunming mice (body weight 18-22 g) were provided by Hubei Provincial Laboratory Animal Public Service Center, and all experiments followed the WHO Guidance of Humane Care and Use of Laboratory Animals. All mice were maintained at room temperature (22–25 °C) with a reversed natural light–dark cycle (12 h of light and 12 h of dark) and had food optionally. The project was reviewed and approved by the scientific committee of the Department of Animal Biology of South-Central University for Nationalities.

The AFLD model was induced by intragastric administration of Erguotou (REDSTAR WINE, Beijing, China, with an alcohol concentration of 56%). The mice (n=8) were given Erguotou orally (BW: equivalent to alcohol 5 g/kg) every 12 hours for a total of three times.

For the first acute experiment, after one week of adaptation, the animals were divided into five groups with a random grouping method: a blank control group, an AFLD model group, low-dose treatment group, high-dose treatment group and a treatment control group. The mice in the model and treatment groups were given Erguotou orally (BW: equivalent to alcohol 5 g/kg) every 12 hours for a total of 3 times. The mice in blank control group was given normal saline orally in the same volume as alcohol. At the same time, the mice were given 0.1 ml or 0.2 ml fresh SE orally in the low-dose and high-dose treatment and treatment control groups. Ethanol and SE were administered by intragastric injection every 12 hours for a total of three times. After the second gavage, fasting was continued. The mice were sacrificed 4 hours after the last gavage, and their blood and liver samples were collected and stored.

For the second acute experiment, different polar solvents extracts were suspended in Erguotou at 10 mg/ml (BW: 100 mg/kg) for medication. With a random grouping method, the animals were divided into six groups: a blank control group, an AFLD model group, and different solvent extract treatment groups: a petroleum ether extract treatment group (PETE group), an ethyl acetate extract treatment group (EACE group), a methanol extract treatment group (MTE group) and an aqueous extract of crude polysaccharide treatment group (ACP group). The mice in the model groups were given Erguotou orally (BW: equivalent to alcohol 5 g/kg). At the same time as the 3rd and 7th intragastric administration, LPS was injected intraperitoneally (BW: 10 mg/kg). The mice in blank control group was given normal saline orally in the same volume as alcohol. The mice in each component treatment group were given a suspension (BW: 0.1 ml/10 g) made of Erguotou and extract by intragastric administration. Mice were given 5 g/kg of alcohol every 12 hours, a total of 9 times, and LPS (BW: 10 mg/kg) was injected intraperitoneally at the 36 h and 84 h. After the 8th gavage, fasting was continued, and the mice were killed 4 h after the last gavage, and their blood and liver samples were collected and stored.

### **Biochemical tests and histological assay**

In the first acute experiment, blood was collected and stored in a refrigerator at 4 °C for 2 h, and then centrifuged at 4 °C and 1000 rpm for 5 min in a refrigerated centrifuge. The resulting serum was stored in a refrigerator at -86 °C for test of serum biochemical indicators. Serum activity levels of triglyceride (TG), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were tested using an automatic biochemical analyzer (FUJIFILM, Tokyo, Japan). The thawed liver was ground into a homogenate, and the level of TG was measured using a Tissue Triglyceride Assay kit (Applygen Technologies Inc., Beijing). Another part of the frozen liver tissue was stained with oil red O, to observe the distribution of lipid droplets in the tissue (the lipid droplets are stained red). In the second acute experiment, the biochemical test of the blood sample was the same as the first experiment. A 1 cm<sup>2</sup> square small piece of liver tissue was cut with a scalpel, placed in glutaraldehyde, frozen and fixed in the dark, and used as a transmission electron microscope to observe mitochondrial changes. The largest lobe of liver tissue was fixed in 10% formalin to prepare paraffin sections for H&E staining.

### **Statistical analysis**

All results are expressed as the means ± standard deviation (SD), and the data were statistically analyzed using one-way ANOVA (GraphPad Prism 5), followed by the Tukey test for post hoc analysis between groups. Statistical significance of difference was determined as  $P < 0.05$ .

## **RESULT**

For the first acute experiment, there were no significant differences in serum ALT and AST between the different groups (Table 1), but serum triglyceride levels were different in each group. The serum triglyceride content of the model group mice was increased significantly compared with that of the blank control group. After SE treatment, the level of triglyceride in the serum of mice decreased. This result indicated that fresh SE could reduce alcohol-induced hyperlipidemia effectively.

**Table 1:** Serum biochemical index of the first acute experiment

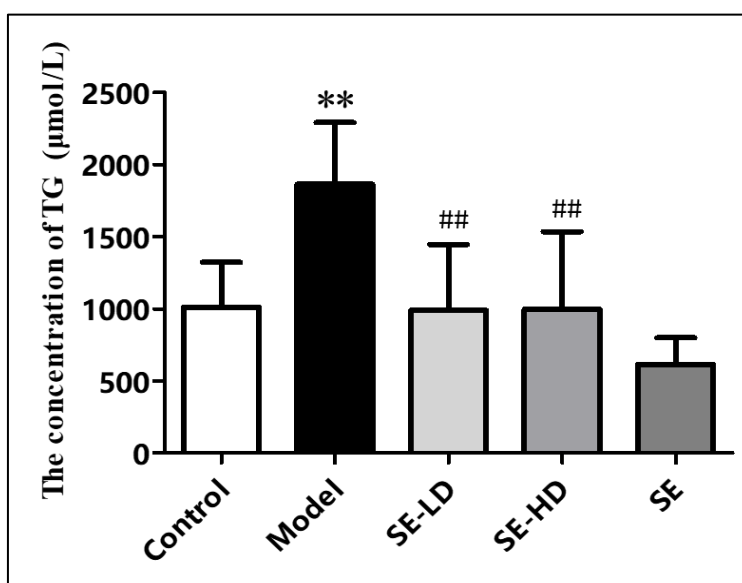
	Control	Model	SE-LD	SE-HD	SE
TG (mg/dl)	146.6±23.0	223.4±98.7*	212.2±51.9	182.5±66.3	171.5±35.4
AST(U/L)	114.0±15.0	110.2±17.4	185.0±185.0	133.8±28.4	124.5±30.2
ALT(U/L)	36.1±6.1	43.1±18.6	44.7±20.5	45.6±16.7	40.8±10.1
AST/ALT	3.2±0.8	2.8±0.7	3.6±1.6	3.1±0.7	3.1±0.6

compared with blank control group mice, \* $p < 0.05$

We tested triglycerides in the liver of mice (Fig. 1), and found that the model group had significantly higher levels of triglycerides in the liver compared with the blank control group ( $p < 0.01$ ). Both low-dose and high-dose SE could reverse that significantly ( $p < 0.01$ ). We observed oil red O staining of liver tissue under a microscope (Fig. 2). The lipid droplets of the model group increased significantly compared with that of the blank control group. In addition, through treatment, lipid droplets

were significantly reduced. That further shows that SE could effectively reduce the lipid accumulation in liver tissue caused by alcohol.

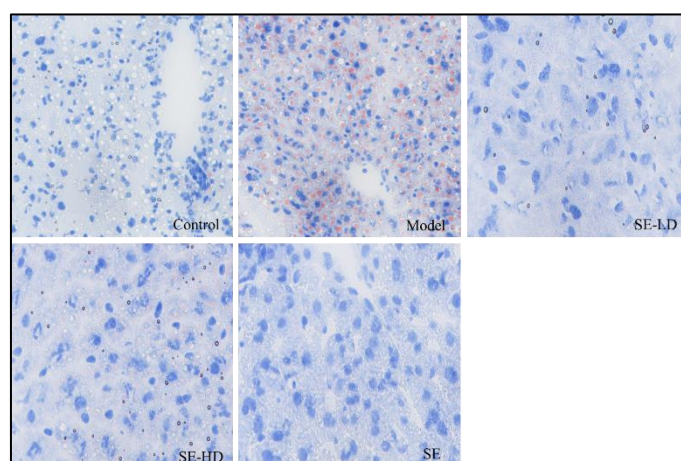
In the second acute experiment, the mortality rate of the animal in the model group was 7/12, and the mortality rate in each treatment group was 1/8, 1/7, 4/8, and 1/8. This result indicated that SE might have an anti-alcoholic hepatitis effect.



**Figure 1:** TG content in liver tissue of the first acute experiment compared with blank control group mice, \* $p < 0.05$

The serum levels of triglycerides in the model group mice were significantly increased compared with that of the blank control group (Table 2). However, the increase in the serum triglyceride level of the mice in each treatment group couldn't be reversed compared with the model group. This indicated that alcohol and LPS co-operation induced

an severe damage in lipid accumulation that the test part couldn't reverse it or the treatment effect on the separate part is weakened than SE.



**Figure 2:** Oil red O staining of the frozen liver tissue section in the first acute experiment

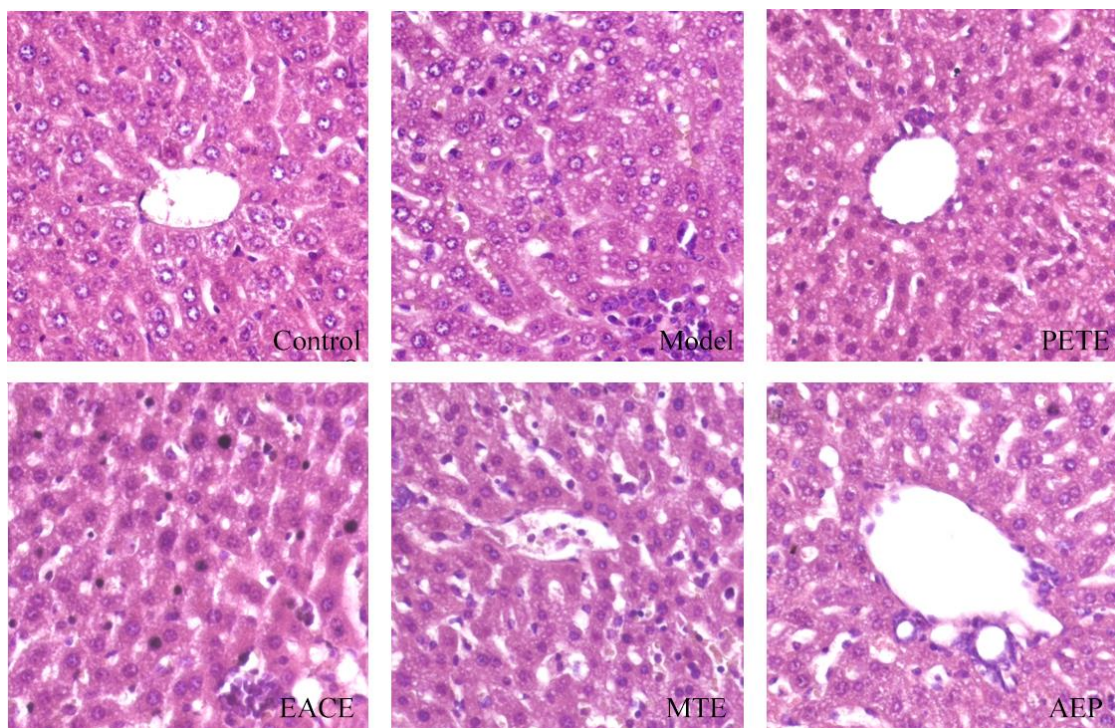
**Table 2:** Serum biochemical index of the second acute experiment

	Control	Model	SE-PETE	SE-EACE	SE-MTE	SE-ACP
<b>TG (mg/dl)</b>	175.63±23.19	272.83±122.23	291.57±110.18	284.50±142.51	260.75±120.68	261.67±130.17
<b>AST (U/L)</b>	102.13±16.44	101.17±21.40	104.71±19.78	94.20±12.21	81.00±20.96	94.50±12.39
<b>ALT (U/L)</b>	34.63±8.75	36.83±7.88	37.00±20.53	32.00±9.82	38.00±25.81	31.50±6.57
<b>AST/ALT</b>	3.06±0.63	2.81±0.65	3.115±0.77	3.14±0.86	2.67±1.48	3.12±0.77

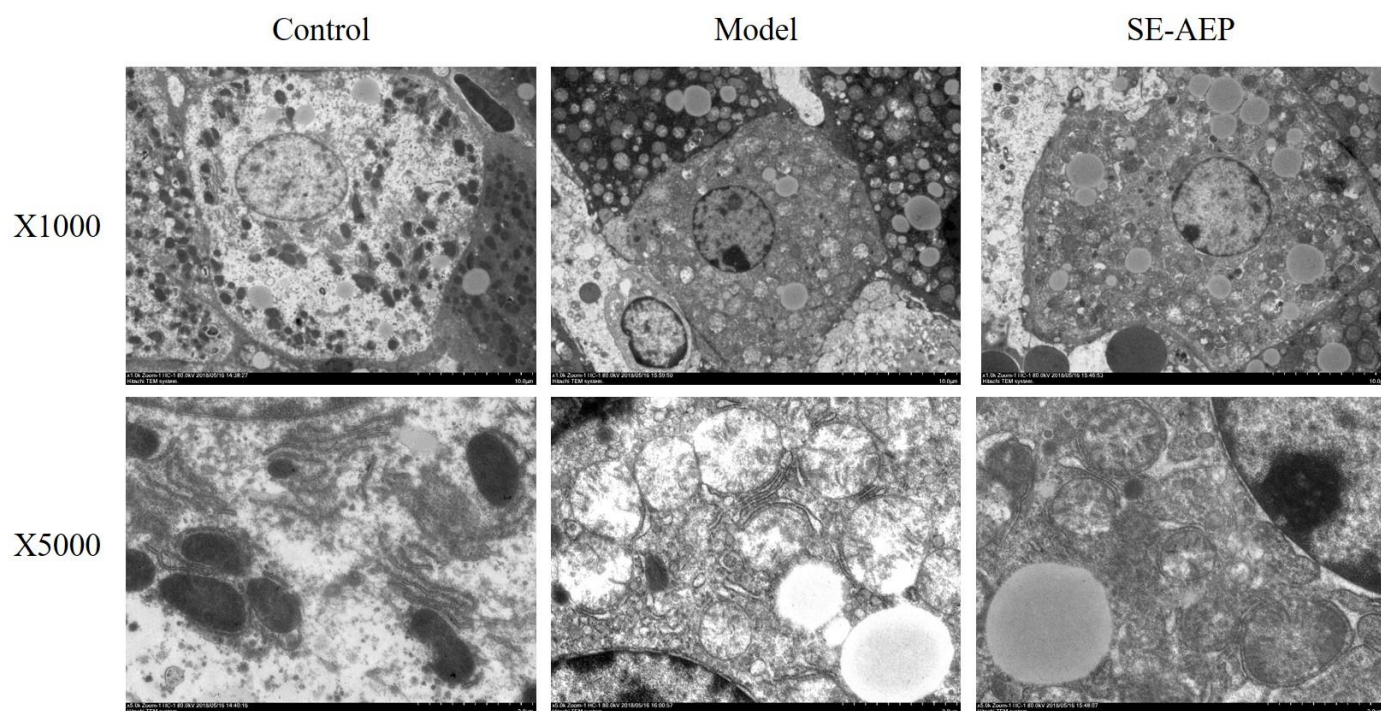
compared with blank control group mice, \*p < 0.05

Compared with the blank control group, the model group had more severe inflammatory aggregation and steatosis in the liver (Fig. 3). Compared with the model group, the liver morphology of mice in each treatment group showed improvement, of which the crude polysaccharide treatment had the best effect. The ultrastructural pathological changes of liver tissues were observed using transmission electron microscopy (Fig. 4). The blank control group had a complete cell morphology, a large number of mitochondria in the parenchymal

cells and a complete structure, and the internal crest was clear. In the model group, large groups of lipid droplets were distributed, and the boundaries of intracellular organelles were blurred, the mitochondrial membrane was ruptured, and the internal ridge disappeared. After treatment with crude polysaccharide, the mitochondrial membrane structure tended to be normal, and the internal ridge structure was generally intact. It indicated that the crude polysaccharide component has a protective effect on mitochondrial damage caused by alcohol.



**Figure 3:** Tissue paraffin section H&E (400X) stain of the second acute experiment



**Figure 4:** Transmission electron microscope observation of liver tissue of the second acute experiment

## DISCUSSION

According to our knowledge, the present study reports the anti-ALD effect of SE for the first time. AFLD is early stage of ALD. In the classic mice ALD model, SE showed a potent to reduce hyperlipidemia and liver lipid accumulation. AH is the next stage of AFLD. AH is more dangerous and cause the main mortality in clinical ALD. Thus, a more severe ALD model was established to investigate whether there is still a protective effect of SE. Alcohol and LPS were given as two strike to simulate the stage between AFLD and AH. After the intervention of extracts from various parts of SE, the mortality rate of all groups except the methanol part was reduced. It is speculated that SE may have an effect against alcoholic hepatitis.

For the early course of AH, alcohol caused an increase in serum triglyceride levels, and extracts from various parts did not reverse well. According to the perspective of histopathology, the polysaccharide was more effective than other components in combating the damage of alcohol and LPS to liver tissues, and alleviated liver fatty and inflammatory infiltration.

The SE polysaccharides reduced the mitochondrial damage, which might reverse the damage of alcohol to mitochondrial aerobic respiration, inhibiting lipid peroxidation in the liver, thereby reducing the accumulation of free fatty acids in the liver, and preventing inflammation gathered further. It also shows that the drug can inhibit liver lipid accumulation and increase serum triglyceride levels in the early stage of the development of alcoholic fatty liver disease. As the course of the disease progresses and the condition worsens, the treatment effect of the drug on elevated serum triglyceride levels becomes poor, but it can still inhibit the fatty tissue of the liver tissue, and can effectively reduce the inflammatory aggregation in the liver tissue and reverse liver damage. It shows that the treatment of ALD should be controlled at the early stage of the development of the disease, and the disease should be avoided in a timely manner.

## CONCLUSION

In conclusion, SE could effectively fight the increase of serum triglyceride level caused by alcohol and the accumulation of lipid in liver tissue and the production of inflammatory foci. Its effective part against early alcoholic hepatitis was polysaccharide.

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## Author Disclosure Statement

The authors declare no conflict of interest.

## ETHICAL CONSIDERATIONS

All applicable international and national guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

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