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Sedum sarmentosum Protects against Alcoholic Fatty Liver Disease in Mice

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ABSTRACT

Objective: This study was carried out to explore the protective effect of *Sedum sarmentosum* (SS) on alcoholic fatty liver disease (AFLD). **Methods:** We conducted an acute experiment in which female Kunming mice were given intragastric administration of alcohol to induce AFLD every 12 h, three times in total. Fresh SS were ground into homogenate for oral treatment, and the mice received ethanol and SS at the same time. The mice were sacrificed 4 h after the last alcohol administration, and serum and liver were collected for testing. We found that SS was effective and then carried out subsequent experiments. The dried SS was ground into powder and successively extracted by petroleum ether, ethyl acetate, methanol and water. The extracts of various polar solvents were used for treatment via intragastric administration. **Results:** The serum triglyceride levels in serum and liver were decreased with the administration of fresh SS. The methanol extract and water fractions had better therapeutic effect. **Conclusion:** Fresh SS can serve as an effective therapeutic of AFLD, and the high polar fractions were active.

Keywords: *Sedum sarmentosum*, alcoholic fatty liver disease, serum triglyceride.

INTRODUCTION

With the extensive application of traditional Chinese medicine, we find that many traditional Chinese medicines can be applied for new pharmacological effects in the process of treating diseases. SS is a common succulent plant and a vegetable. Its roles in promoting diuresis and treating jaundice and carbuncle swollen sores have been documented in the *Chinese Pharmacopoeia*. SS has been shown to inhibit hepatocyte damage caused by lipid peroxidation, thus providing adjuvant therapy for chronic hepatitis [1]. The aqueous extract has a strong inhibitory effect on the proliferation of human hepatoma cells [2]. The flavanones from SS can significantly attenuate CCl_4-induced liver fibrosis in rats [3], indicating that SS targets and protects the liver. Alcoholic liver disease (ALD) is associated with high morbidity and mortality worldwide, with effects ranging from fatty liver to hepatitis, necrosis, progressive fibrosis and hepatocellular carcinoma [4]. ALD may be caused by alcohol-induced oxidative stress and an increase in inflammatory factors in the liver, which may affect the process of lipid metabolism and ultimately lead to liver damage [5-8]. SS was commonly used as a vegetable or for the treatment of hepatitis by the native Tujia ethnic group when we surveyed in Badong County, Hubei Province. Considering its existing mechanism of action, we asked whether it can be applied to AFLD.

MATERIALS AND METHODS

Collection, identification and preparation of the plant material

Fresh herbs of *Sedum sarmentosum* 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 SS was cleaned, drained and ground with normal saline (0.3 g: 1 ml) into homogenate for the first acute experiment, and other samples were dried and powdered for the second acute experiment.

Extraction procedure

Dried powder (600 g) was extracted by heat reflux for 2 hours in 2.5 L 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, 21 g), ethyl acetate extract (EACE, 47 g), methanol extract (ME, 85.6 g), and aqueous extract (AE, 102.7 g) and were stored 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 ℃) 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 four groups with a random grouping method: a blank control group, an AFLD model group, a 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 three doses. Mice in the blank control group were given normal saline orally in the same volume as alcohol. At the same time, the mice were given 0.2 ml fresh SS orally in the treatment and treatment control groups. Ethanol and SS were administered by intragastric injection every 12 hours for a total of three doses. After the second administration, the mice were fasted and were then sacrificed four hours after the third administration. Blood and liver samples were immediately collected.

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 treatment group (AE group). After three doses, the mice were sacrificed. We also collected blood and liver samples to detect biochemical and pathological indicators.

Sarmentosin is a compound of cyanogenic glycosides extracted from SS that has a positive effect on liver function in intrahepatic cholestasis rats[9]. In the third acute experiment, to investigate whether sarmentosin is an effective component for the treatment of AFLD, the animals were divided into three groups: a blank control group, an AFLD model group and a sarmentosin treatment group. The mice were given sarmentosin orally (*Chem Faces* Biochemical co., LTD, Wuhan, China) (BW: 10 mg/kg) dissolved in normal saline for medication immediately following Erguotou administration (BW: equivalent to alcohol 5 g/kg). After three doses, the mice were sacrificed. Blood and liver samples were collected.

Biochemical tests and histological assay

In the first acute experiment, blood samples were centrifuged at 4 ℃ to produce serum. The serum activity levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglyceride (TG) and alkaline phosphatase (ALP) were tested using an automatic biochemical analyzer (FUJIFILM, Tokyo, Japan). The liver tissue was cryopreserved for use. The frozen liver tissue was cut into frozen sections for oil red O staining to observe the distribution of lipid droplets in the tissue (the lipid droplets were stained red, and the nuclei were stained blue). The thawed liver tissue was ground into homogenate, and the lysate was subjected to triglyceride content analysis using a Tissue Triglyceride Assay kit (Applygen Technologies Inc., Beijing).

In the second acute experiment, blood samples were centrifuged at 4 ℃ to produce serum for biochemical testing. The largest lobe of liver tissue was fixed in 10% formalin to prepare paraffin sections for H&E staining. The remaining lobes were ground into homogenate for triglyceride content analysis.

In the third acute experiment, blood samples were centrifuged at 4 ℃ to produce serum, and the serum activity levels of ALT, AST, TG and ALP were tested. The liver tissue was used for triglyceride content analysis.

Statistical analysis

All results are expressed as the means ± standard deviations (SDs), 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 $P < 0.05$.

RESULTS

For the first acute experiment, the serum biochemical indicators (Table 1) ALT, AST, and ALP were not significantly different in the different groups, but the serum triglyceride levels were different in each group. The serum triglyceride in the model group was significantly increased ($P < 0.05$) compared with the blank control group. After being treated with fresh SS, the serum triglyceride levels decreased, indicating that fresh SS can effectively reduce alcohol-induced hyperlipidemia.

We observed oil red O staining of liver tissue under the microscope and quantified lipid droplets using a Nuance Multispectral Imaging System (Cambridge Research and Instrumentation Inc., Woburn, MA) (Figs. 1 and 2). There was a clear increase in lipid droplets in the AFLD model group compared with the blank control group ($P < 0.01$). Moreover, lipid droplets were significantly decreased ($P < 0.05$) by treatment. Similarly, we concluded that SS can effectively reduce liver fat accumulation caused by alcohol. We also tested the triglyceride levels in the liver (Fig. 3), and the results further conformed the antihyperlipidemic effect.

In the second acute experiment, there were no significant differences in AST, ALT, and ALP levels between the groups (Table 2), consistent with the previous experiment. The serum triglyceride levels of the AFLD model group were increased significantly compared with the blank control group ($P < 0.001$). However, PETE and EACE were not able to lower the triglyceride contents in the serum. In fact, EACE increased the triglyceride level in the serum. In contrast, MTE ($P < 0.05$) and AE ($P < 0.05$) reduced the triglyceride levels in serum. The results of triglyceride content testing showed that the aqueous extract can effectively reduce triglyceride accumulation liver tissue ($P < 0.001$) (Fig. 4). From H&E staining (Fig. 5), we observed that the liver tissue morphology was intact in the blank control group but had changed greatly in the AFLD model group, including multiple necrotic areas, inflammatory foci and adipose. In the treatment groups, PETE and EACE neither decreased serum triglyceride levels nor reduced lesions in tissues caused by alcohol; however, MTE and AE both decreased serum triglycerides, and AE could protect against alcohol-induced liver damage more effectively.

In the third acute experiment, there were no significant differences in AST, ALT, and ALP levels between the groups (Table 3). Serum triglycerides in the AFLD model group were increased compared with the blank control group and were decreased in the sarmentosin treatment group, but there were no significant differences. Unexpectedly, the triglyceride contents in the liver were increased ($P < 0.05$) significantly in the treatment group compared with the model group (Fig. 6). Therefore, it could not be concluded that sarmentosin was the active ingredient in SS.

DISCUSSION

Only a few studies have focused on the active competent of SS. Since sarmentosin was supposed to be the active ingredient of SS for chronic hepatitis treatment, its antihyperlipidemic effect was detected in an alcohol-induced fatty liver model. However, sarmentosin could not reduce the lipid accumulation in the liver caused by alcohol.

The total flavone contents were detected in the methanol extract and aqueous extracts of SS by the spectrophotometry with rutin as the control sample. The total flavonoid contents were 2.38% and 1%, respectively. However, the AE was more efficient than the MTE, and the total flavonoids might not be the main ingredient responsible for the anti-ALFD effect. Further investigations will be required to identify the real active ingredients.

CONCLUSION

In conclusion, SS could protect against alcohol-induced hyperlipidemia and lipid accumulation in the liver, and the high polar fractions were active. The well-known compound sarmentosin was not the active ingredient.

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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.

Table 1: Serum biochemical index of the first acute experiment

	Blank control group	ALD model group	Treatment group	Treatment control group
GOT (U/L)	107.8±24.5	101.4±39.9	124.6±78.0	92.6±8.0
GPT (U/L)	39.0±14.2	40.3±10.3	49.6±24.3	38.0±5.0
GOT/GPT	2.9±0.5	2.5±0.7	2.5±1.0	2.5±0.3
TG (mg/dL)	100.4±18.6	199.8±117.4*	167.0±87.2	101.38±40.37
ALP (U/L)	512.6±184.7	459±146.8	459.8±139.4	456.7±79.3

compared with blank control group mice, *p < 0.05

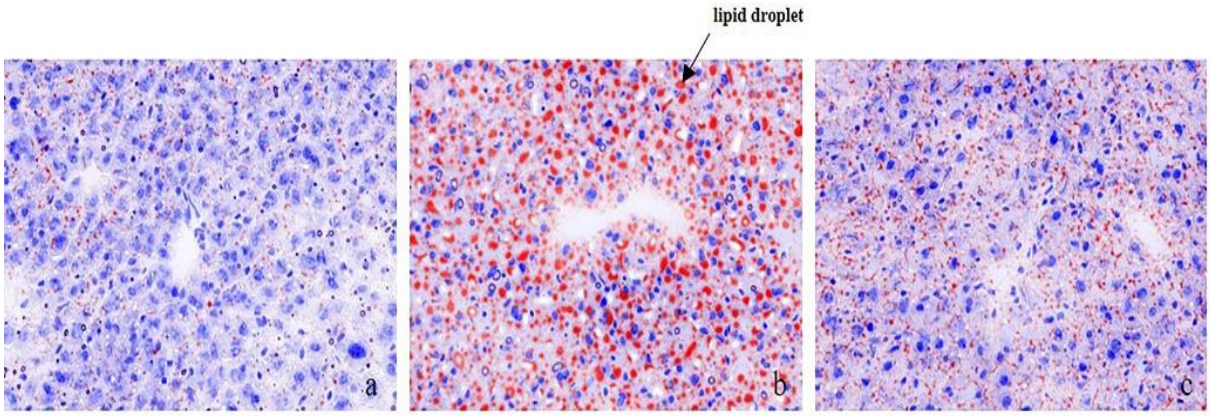

Figure 1: Frozen tissue section oil red O stain of the first acute experiment *a: Blank control group b: AFLD model group c: Treatment group*

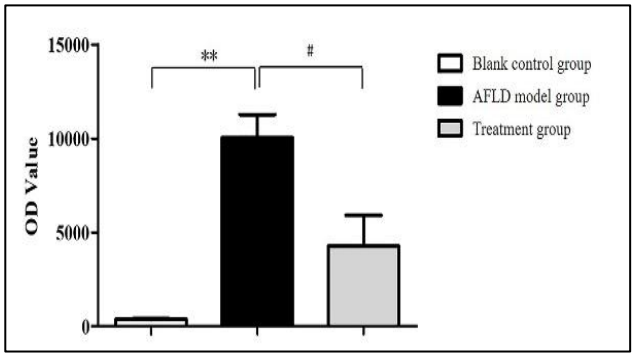

Figure 2: Multi-spectral imaging to quantify lipid droplets in oil red O stain *compared with blank control group mice, **p < 0.01 compared with AFLD model group mice, #P<0.05*

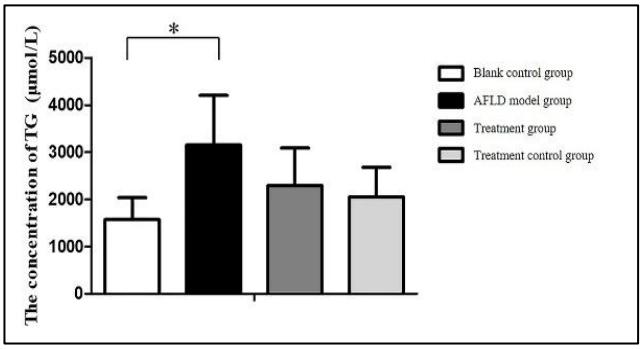

Fig 3: TG content in liver tissue of the first acute experiment *compared with blank control group mice, *p < 0.05*

Table 2: Serum biochemical index of the second acute experiment

	Blank control group	ALD model group	PETE group	EACE group	MTE group	AE group
GOT (U/L)	139±28.78	145.5±26.82	155±20.11	156.83±16.61	131.67±37.73	123.6±21.61
GPT (U/L)	51.5±12.79	56.33±7.23	61.5±11.1	56±17.46	48.83±7.73	44.6±14.28
GOT/GPT	2.85±0.97	2.57±0.23	2.56±0.4	2.93±0.51	2.7±0.58	2.93±0.77
TG (mg/dl)	111±16.47	380.25±94.90***	295.71±130.86	398.67±99.61	168±54.62#	184.2±73.72#
ALP (U/L)	194.83±40.95	261.29±43.26	230.43±49.75	259.14±64.75	251.57±56.72	226±47.84

*compared with Blank control group mice, ***P < 0.001*

compared with AFLD model group mice, #P<0.05

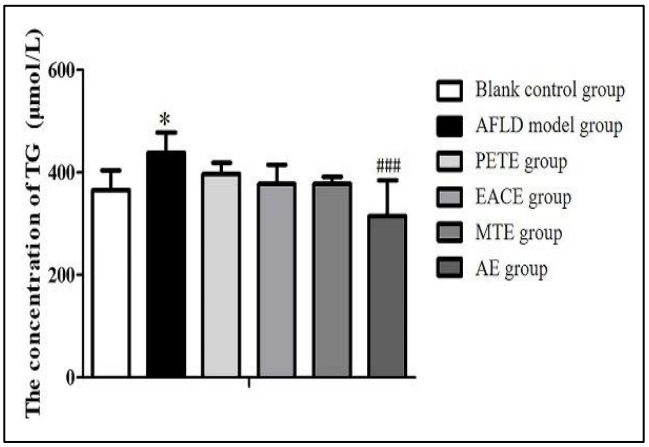

Figure 4: TG content in liver tissue of the second acute experime *compared with Blank control group mice, *P < 0.005 compared with AFLD model group mice, ###P<0.001*

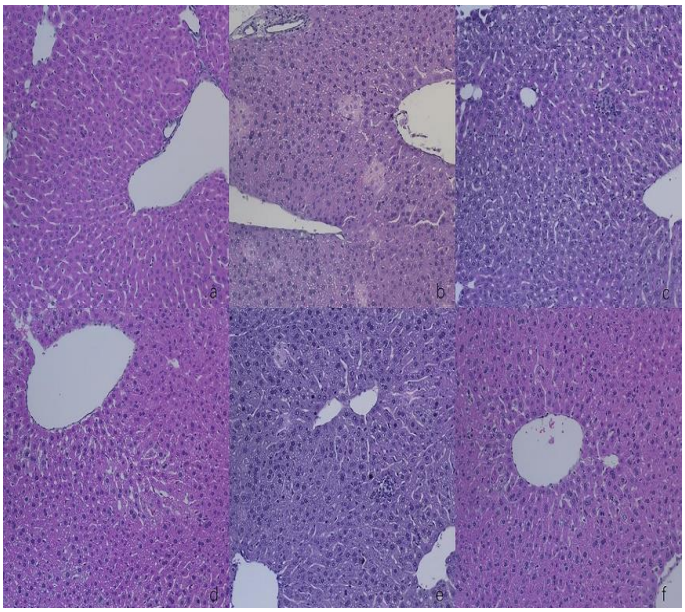

Figure 5: Tissue paraffin section H&E (400X) stain of the second acute experiment *a: Blank control group; b: AFLD model group; c: PETE group; d: EACE group; e: MTE group; f: AE group*

Table 3: Serum biochemical index of the third acute experiment

	Blank control group	AFLD model group	Sarmentosin treatment group
AST(U/l)	168.75±63.53	156.13±29.22	164.25±32.27
ALT(U/l)	37.13±10.60	48.75±18.70	42.25±11.78
AST/ALT	4.49±0.95	3.49±1.15	3.79±0.90
TG(mg/dl)	116±37.08	205.38±132.31	177.25±61.79
ALP (U/l)	238.63±130.44	147.75±53.57	181.25±55.37

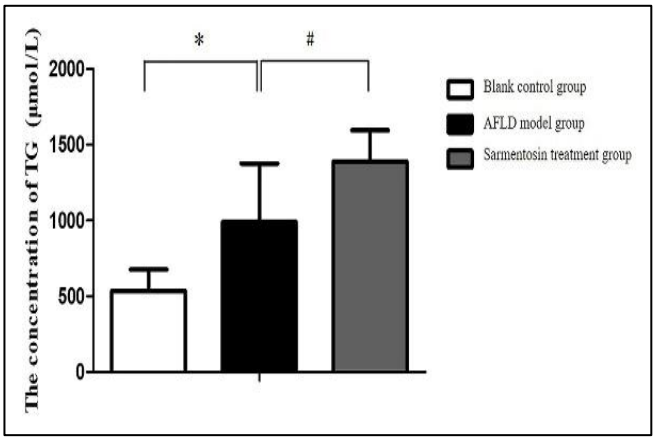

Figure 6: TG content in liver tissue of the third acute experiment *compared with Blank control group mice, *P < 0.005 compared with AFLD model group mice, #P<0.001*

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