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Full Length Research Paper

Hepatoprotective and toxic characteristics of the whole herb of traditional Tibetan folk medicine *Swertia mussotii* Franch.

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***Swertia mussotii* Franch. (SM) (Gentianaceae) was a traditional Tibetan folk herb for hepatitis in the Qinghai-Tibet plateau areas. For understanding the hepatoprotection and toxicity characteristics of *Swertia mussotii* Franch., the whole plant of *Swertia mussotii* was extracted by water (SME-b) and 75% ethanol (SME-a); then, SME-a was dissolved with water, the supernatant was SME-c, and the sedimentation was SME-d. 17.76% SME-a, 14.48% SME-b, 10.13% SME-c, and 4.35%SME-d were extracted from *Swertia mussotii*. These four extracts were evaluated with acute toxicity and hepatoprotective effect on CCl_4-induced acute liver damage in mice. The toxic material mainly reside in SME-c and SME-a. The hepatoprotective materials were in SME-a, SME-c and SME-d, while SME-b had no hepatoprotective activities. So, SME-a and SME-c had both the hepatoprotection and toxicity characteristics; SME-b had no toxicity or the hepatoprotection characteristics; only SME-d was hepatoprotective function without toxicity, which could be a good candidate for new drug healing the live disorder.**

Key words: *Swertia mussotii* Franch., Gentianaceae, Tibetan herb, extracts, hepatoprotective activity, toxicity.

INTRODUCTION

Liver is the organ for metabolism and detoxification of various components enter into the body. The toxins and drugs, viral infections (Hepatitis A, B, C, D, etc.) and microbial infections of *Entamoeba histolytica* can cause damages to the hepatocytes (Sharma and Ahuja, 1997). Now, liver diseases are very common and large public health problems in the world, which give a big challenge to the modern medicine. Plant-based traditional medicines are widely and successfully used in the treatment of liver disorders, for example, *Picrorhiza kurroa* (Chander et al., 1992), *Phyllanthus emblica* (Gulati et al., 1995), *Silybum marianum* (Flora et al., 1998).

Swertia mussotii Franch is a biennial herb, which has been used to treat the febrile diseases in liver and gallbladder in Tibetan traditional medicine(Yang, 1991). Because of good treatment, people had pay attentions on this plant for a long time. It belongs to the family of Gentianeceae, the genera of Swertia (Yang, 1991). Naturally, it exists strictly on the high alpine lands of the Qinghai-Tibet Plateau, at altitudes ranging between 3200 and 3800 m (Yang et al., 2005). Artificial cultivation of SM was established in 2005, for obtaining more resources (Chen et al., 2005). Chemical analysis discovered SM contained swertiamarin, sweroside, gentiopicroside, mangiferin and oleanolic acid (Sun et al., 1991; Zhang et al., 2009; Sun and Ding, 1981). These constituents had been proved to be hepatoprotective (Liu et al., 1995; Kondo et al., 1994; Lv et al., 2004). But, the content of these constituents were not abundant in SM (Song et al., 2004; Ma et al., 2005; Yang et al., 2005), hence, the hepatoprotection mechanism of SM can not been explained completely.

Modern pharmacological researches have shown SM can repair the fibrillation of the liver (Han et al., 2009; Han et al., 2008), exhibit the protective effect against liver damage induced by hypoxia in rats (Du et al., 1983), the CCl_4-induced liver damage in mice (Luo et al., 2008) and

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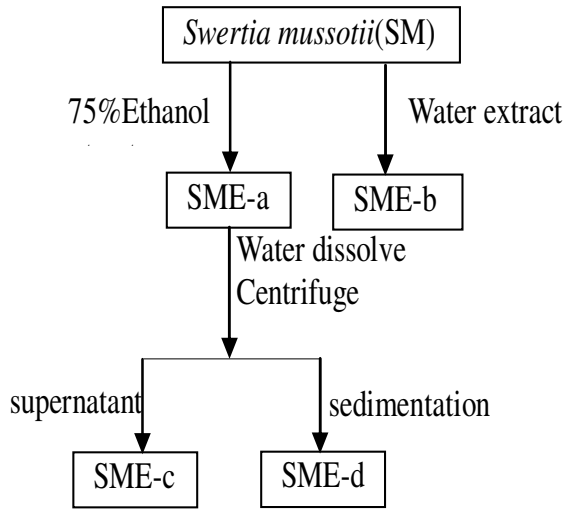

Figure 1. The procedure chart of extract process.

the immunological liver injury in mice (Xu, 2008). But in these pharmacological researches, only the one step extract were studied, and the toxicity evaluation was no performed. The present manuscript describes the preparation of 75% ethanolic and aqueous extractsof the whole plant and evaluation of acute toxicity.

MATERIALS AND METHODS

Plant material

The whole plant of SM was collected at the full-blooming stage in July 2008 from Sichuan province, China. It was authenticated by the centre of Tibetan Medicine, Northwest Institute of Plateau Biology, CAS. The whole plant of SM was dried under shade and cut into small pieces before extraction.

Chemicals

Carbon tetrachloride (CCl_4), medical alcohol (>95%) and other solvents used were purchased from Xining Kexi Company (Xining, China).

Extraction

Four extracts were obtained according to the procedure chart in Figure 1. 1 g of crude SM was subjected to continue hot extraction by using 10 ml ethanol (75% v/v) for three times, and the 75% ethanol extract was token as SME-a; 1g of crude SM was subjected to continue hot extraction by using 10 ml distilled water for three times, and the aqueous extract was SME-b; 1 g SME-a was dissolved in 8ml distilled water, then the supernatant was token as SME-c, and the sedimentation was token as SME-d. The ethanol and the most water of each extract were removed by distillation under reduced pressure. A little sample extracts were dried on the water bath for calculating water content of every extracts. At end, these extracts were suspended in 0.5% sodium carboxymethyl cellulose and used for the present experiments.

Experimental animals

KM mice (male and female) 20 - 25 g were purchased from Laboratory Animal Center, Gansu College of Traditional Chinese Medicine. The animals were maintained at a constant temperature of 23 ± 2℃ and fed with standard laboratory chow (Beijing keaoxieli feed Co., LTD, Beijing, China) and tap water. Experiments were performed based on animal ethics guidelines of Institutional Animal Ethics Committee.

The acute toxicity evaluation

The acute toxicity of the extract (SME-a, SME-b SME-c and SME-d) was determined in mice as described by Lorke (1983).

The hepatotoxicity evaluation

In this study, normal groups were treated with 20 ml/kg distilled water for 8 days orally; control groups were treated the same as normal group. Test groups were treated with half dose of LD_{50} or MDT of SME-a, SME-b, SME-c, SME-d. Each group was composed of ten mice. On the 8^{th} day, CCl_4 10 ml/kg (0.1%, v/v in olive oil) was administrated by intraperitoneal injection to all groups except normal groups. At 22 h after the last dose, the blood of all mice was collected for measuring the alanine aminotransferase (ALT) value (Drotman and Lawhorn, 1978).

The estimation of blood biochemistry

Serum activities of ALT were used as biochemical markers for the acute hepatic injury. Serum was separated by centrifuging at 3000 rpm for 10 min and used for the estimation of serum ALT. ALT activities were determined by commercially available kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Statistical analysis

All statistical analyses were performed by using Microsoft Excel 2000 or the SPSS 10.0 for windows software package. The date were analyzed by Student's t-test to assess the significance of the differences between two means or by one-way analysis of variance (ANOVA) followed by least-significant-difference (LSD) test for more than two means (Milton, 1983). Statistical significance was considered at $p < 0.05$.

RESULTS

The contents of SME-a, SME-b, SME-c and SME-d

According to the dry weight of SM, SME-a, SME-b, SME-c and SME-d, the contents of SME-a, SME-b, SME-c, and SME-d extracted from SM were calculated. SM contains 17.76% SME-a, 14.48% SME-b, 10.13% SME-c, and 4.35% SME-d.

The acute toxicity of extracts

The LD_{50} values of SME-a and SME-c are 27.24 g/kg body weight (BW) and 20.2 g/kg BW. Limited to the solubility of the extracts, the LD_{50} of SME-b, SME-d can

Table 1. The content, acute toxicity evaluation and hepatoprotective activity of four extracts.

Extracts	SME-a	SME-b	SME-c	SME-d
Percentage (%)	17.68	14.23	13.34	4.33
SME LD_{50}/MTD (g/kg BW)	27.24 (LD_{50})	40.00 (MTD)	20.20 (LD_{50})	14.28 (MTD)
Delivery dose (g/kg BW)	13.62	20	10.1	7.14
ALT reduction percentage (%)	60.5	3.8	59.2	77.3
Average reduction percentage of ALT (%/g)	4.4	0.2	5.9	10.8

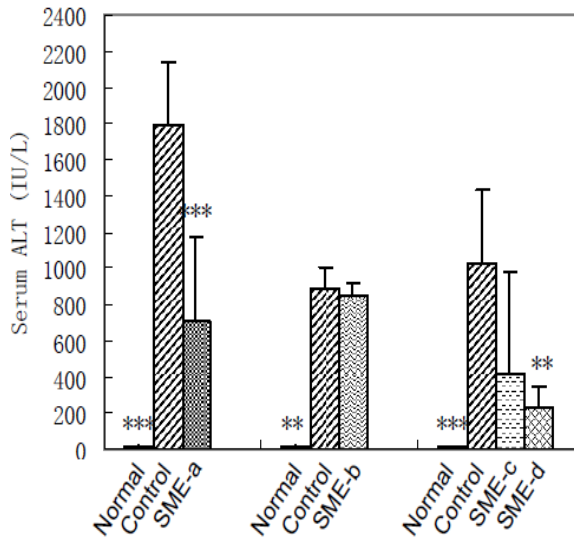

Figure 2. The ALT activity in experiments for the hepatoprotective activity evaluation. The ALT value is indicated as mean ± S.D. (n = 10), significantly different from control, *p < 0.05, **p < 0.01, ***p < 0.001.

not be obtained, and MTD of them are 40 g/kg BW, 14.28 g/kg BW respectively (Table 1).

The hepatoprotective activity

Serum activity of ALT is one of the most sensitive biochemical indicators for the CCl_4-induced acute liver injury in mice (Drotman and Lawhorn, 1978). So, it was employed in this research. The change of the ALT activities and the capabilities of the extracts on decreasing ALT were showed in Figure 2 and Table 1. Comparing with the normal group, the serum ALT activities of the control treated with CCl_4 were significantly elevated ($p < 0.001$). The four extracts of SM all can reduce the level of serum ALT in mice treated with CCl_4. The SME-a and SME-d can decrease significantly the serum ALT activities. The effects of SME-b and SME-c were not apparent statistically. Hepatoprotective activity of SME-d was the strongest, which could reduce 77.3% of the ALT in mice treated with CCl_4, while SME-a, SME-b, SME-c were 60.5, 3.8 and 59.2% respectively.

Comparing their average reduction percentages, the hepatoprotective effect of SME-d would be further effective than SME-a, SME-b, SME-c (Table 1).

DISCUSSION

Swertia mussotii Franch, referred to as “Zang Yin Chen” in Chinese, is an important traditional Tibetan folk medicine and widely used for a long time in Qinghai-Tibetan plateau area. Many researchers are being absorbed to study the active constituents or extracts of SM, for developing the new drug against the hepatitis.

SME-a (the 75% alcohol extracts of *Swertia mussotii*) possessed the hepatoprotective activity, which was consistent with previous findings (Luo et al., 2008, Xu, 2008). But SME-a was not a good candidate for new drugs, because the LD_{50} value of SME-a is 27.24 g/kg, which would be difficult to control the safety of the clinical dose. This should be the first time of reporting the toxicity in SM. In contrast with SME-a, SME-b (aqueous extract) have no remarkable hepatoprotective activity or acute toxicity. SME-a was compose of SME-c and SME-d, so the ALT reduction percentage value of SME-a (60.5%) is between SME-c (59.2%) and SME-d (77.3%). It is interesting that SME-d lost toxicity, which let SME-d be a good candidate for new drug healing the live disorder, while SME-c inherit the toxicity of SME-a. So the toxic and hepatoprotective materials of SM can be separated.

A good candidate for new drug was obtained in this article, but the toxic and hepatoprotective compounds of SM were not isolated. So further research will focus on isolating the toxic and hepatoprotective compounds, and evaluate the possibility of SME-d for new drug dealing the live damages.

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