

Effect of Jiaosanxian on Reducing Blood Lipid

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Abstract: **Objective** To investigate the effects of Jiaosanxian (JSX, consisted of stir-baked *Hordei Fructus Germinatus-Crataegi Fructus-Massa Fermentata Medicinalis*) on reducing blood lipid. **Methods** The model of hyperlipidemic mice was established by feeding high-fat diet. Kunming mice were randomly divided into six groups: blank, hyperlipidemic, Zhibituo, low-, mid-, and high-dose [100, 200, and 400 mg/(kg·d)] JSX groups, and were continuously ig administered for 28 d. The contents of total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), and high density lipoprotein cholesterol (HDL-C) in serum and liver and liver index were determined. **Results** The contents of TC, TG, and LDL-C in serum or liver were decreased significantly but the contents of HDL-C were increased more significantly in the mid- and high-dose JSX groups than those in hyperlipidemic group. The weight and liver index were decreased significantly with the dose increasing of JSX. The lipid-decreasing effects were improved with the dose increasing. **Conclusion** These results suggest that JSX has the significant effects on hyperlipidemia. It could provide the experimental basis for the clinical use of JSX for the treatment of hyperlipidemia.

Key words: cholesterol; hyperlipidemia mice; Jiaosanxian; low-density lipoprotein cholesterol; reducing blood lipid; triglyceride

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Introduction

There is a high proportion of hyperlipidemia in Chinese population. Prevalence of hyperlipidemia in those aged 18 and over was 18.6%. The abnormal blood lipid is at a higher level, moreover, more and more young people are suffering from it, and the incidence of hyperlipidemia has no obvious difference compared with that of middle-aged people (Wen *et al.*, 2005). Hyperlipidemia could bring about atherosclerosis and other cardio-cerebrovascular diseases (Ishikawa, Stokes, and Zhang, 2004; Taniyama and Griendling, 2003), so the research and development of drug with inhibitory effect on hyperlipemia is an important research topic in current medical field. Hawthorn (*Crataegi Fructus*) and malt (*Hordei Fructus Germinatus*) are edible Chinese materia medica (CMM). Hawthorn, which is tasted acid and sweet, little warm in property and channel tropism of the spleen, stomach, and liver (Huang, 2002), has the

functions of strengthening spleen and appetizing. The modern studies show hawthorn has the functions of reducing blood lipid, lowering blood pressure, regulating lipid metabolism, falling blood pressure, antitumor, and anti-oxidation (Liu *et al.*, 2009; Xie, Sun, and Liu, 2009; Hang, 2001; Dong *et al.*, 2009; Li, Guo, and Kang, 2009; Jin and Liu, 2007). Burnt malt which had the function of promoting digestion was obtained by roasting malt. Medicated leaven (*Massa Fermentata Medicinalis*), which is tasted sweet and pungent, warm in property, and channel tropism of the spleen and stomach, has the function of promoting digestion and checking diarrhea (Gao and Jiao, 2002). Medicated leaven contains digestive enzymes, yeast, ergosterol, vitamin complex, naphtha, glycosides, and other substances (Liu and Liu, 2009). Jiaosanxian (JSX, consisted of stir-baked *Hordei Fructus Germinatus-Crataegi Fructus-Massa Fermentata Medicinalis*)

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could remove stagnated food and promote digestion, and be widely used for the treatment of indigestion diseases in clinic. At present, there is no report on the effects of JSX for reducing blood lipid. In the present study hyperlipemic mice models were used to study the lipid-decreasing effects of JSX, hoping to provide a new theoretical basis for the further experimental study and clinical application.

Materials and methods

Reagents and instruments

Hawthorn, malt, and medicated leaven were purchased from Xingyuanchun Drug Store (Guangzhou, China). Zhibituo (ZBT) was purchased from Di'ao Group (Chengdu, China). Cholesterol and sodium cholate were purchased from Sigma-Aldrich Co. LLC (St Louis, USA). Serum total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) test kits were purchased from Nanjing Jiancheng Bioengineering Institute. YP2001N Electronic Balance was purchased from Shanghai Precision Scientific Instrument Co., Ltd. 752 Ultraviolet-visible Spectrophotometer was purchased from Shanghai Precision Scientific Instrument Co., Ltd. EBA21 Centrifuge was purchased from Hettich Co., Ltd. (Germany).

Animals

Kunming mice (SPF grade), weighing (22 ± 2) g, were obtained from Guangdong Laboratory Animal Breeding Center (Guangzhou, China). Each animal was housed in separated cage at temperature (22 ± 0.5) °C and the relative humidity $\geq 40\%$.

Preparation of JSX solution

Malt was stir-baked by the low-degree fire until yellow, then sprayed with a small amount of water, and dried at 80 °C to obtain the burnt malt. Hawthorn was roasted by strong fire until external surface black and the internal russet, sprayed with a small amount of water, and dried at 80 °C to obtain the burnt hawthorn. The burnt medicated leaven was also obtained by the same way. Crushed burnt malt, burnt hawthorn, and burnt medicated leaven were obtained, respectively, and passed through 120-mesh sieve. JSX was prepared by well-mixing the burnt malt, burnt hawthorn, and burnt medicated leaven (mass ratio 1:1:1). JSX

suspension (10, 20, and 40 mg/mL) and ZBT suspension (5 mg/mL) were prepared with carboxymethyl cellulose water solution.

Grouping and administration

Kunming mice were randomly divided into six groups (12 in every group), control, hyperlipemic, ZBT (main component is monascus), and low-, mid-, and high-dose JSX groups. The mice in the control group were fed with basic diet, and those in other groups were fed with high-fat diet (2% cholesterol, 10% lard, and 0.5% sodium cholate). The mice in each group were continuously fed for 28 d, then the appetite and weight of mice were recorded every 2 d. The mice in ZBT group were ig administered with ZBT (50 mg/kg) every day; The mice in low-, mid-, and high-dose JSX groups were ig administered with JSX suspension (10, 20, 40 mg/mL) at different doses (100, 200, and 400 mg/kg) every day; The mice in the control and hyperlipemic groups were ig administered with normal saline at the same volume every day.

Determination of blood lipid and liver lipid

After the last administration, the mice were fasted overnight, the eyeball was taken for blood next day, and the serum was separated and preserved at -20 °C. Then their livers were harvested, washed with normal saline, dried, weighed, and preserved at -20 °C. The contents of TC, TG, LDL-C, and HDL-C in serum and liver were determined according to kit specification. The liver index of mice was calculated by the following formula.

$$\text{liver index} = \frac{\text{liver weight (g)}}{\text{mice weight (kg)}}$$

Statistical analysis

All data were expressed as $\bar{x} \pm s$, and the statistical analysis was performed with software SPSS12.0. Inter-group comparisons were performed with paired *t*-test, and $P < 0.05$ was considered as statistical significance.

Results

Weight and liver indexes

The weight had no statistical difference among different groups before the experiment. The intake had no statistical difference among different groups during the experimental period. The weight was increased significantly in the hyperlipemia group than that in the control group ($P < 0.01$). The weight and liver indexes

were decreased significantly with dose increasing of JSX; There was no statistical difference between the high-dose JSX group and ZBT group (Table 1).

Determination of serum lipid

The contents of TC, TG, and LDL-C were increased but the content of HDL-C was decreased more significantly in the hyperlipemia group than those in the control group ($P < 0.01$), which meant the hyperlipemic model was established successfully. The contents of TC, TG, and LDL-C were decreased significantly but the contents of HDL-C were increased

more significantly in the mid- and high-dose JSX groups than those in the hyperlipemic group. There was no statistical difference between the high-dose JSX and ZBT groups (Table 2).

Determination of liver lipid

The contents of TC and TG in liver were decreased as doses increased. The contents of TC and TG were decreased more significantly in the mid- and high-dose JSX groups than those in the hyperlipemia group. There was no statistical difference between the high-dose JSX and ZBT groups (Table 3).

Table 1 Effect of JSX on body weight and liver indexes in mice ($\bar{x} \pm s$, $n = 12$)

Groups	Dose / (mg·kg ⁻¹)	Body weight / g		Liver indexes / (g·kg ⁻¹)
		day 1	day 29	
control		21.12 ± 1.13	34.57 ± 1.74**	34.9 ± 2.6**
hyperlipemia		21.36 ± 1.64	39.25 ± 2.02	41.1 ± 3.1
ZBT	50	21.47 ± 1.46	35.63 ± 1.85**	36.8 ± 2.7**
JSX	100	21.51 ± 1.42	37.59 ± 1.91	39.0 ± 3.4
	200	21.26 ± 1.38	37.13 ± 1.73*	38.6 ± 2.0*
	400	21.45 ± 1.40	35.74 ± 1.52**	37.9 ± 1.8**

* $P < 0.05$ ** $P < 0.01$ vs hyperlipemic group, same as below

Table 2 Effect of JSX on serum lipid contents ($\bar{x} \pm s$, $n = 12$)

Groups	Dose / (mg·kg ⁻¹)	Contents / (mmol·L ⁻¹)			
		TC	TG	LDL-C	HDL-C
control		2.87 ± 0.51**	1.04 ± 0.27**	0.90 ± 0.31**	2.36 ± 0.29**
hyperlipemia		4.80 ± 0.96	1.68 ± 0.67	1.71 ± 0.54	1.87 ± 0.50
ZBT	50	3.89 ± 0.77**	1.20 ± 0.33**	1.01 ± 0.59**	2.34 ± 0.35**
JSX	100	4.74 ± 0.89	1.56 ± 0.31	1.61 ± 0.45	1.96 ± 0.47
	200	4.09 ± 0.66*	1.25 ± 0.24*	1.20 ± 0.46*	2.27 ± 0.43*
	400	3.90 ± 0.75**	1.23 ± 0.31**	1.05 ± 0.39**	2.30 ± 0.36**

Table 3 Effect of JSX on liver lipid contents ($\bar{x} \pm s$, $n = 12$)

Groups	Dose / (mg·kg ⁻¹)	TC / (mmol·g ⁻¹)	TG / (mmol·g ⁻¹)
control		1.57 ± 0.56**	9.20 ± 1.38**
hyperlipemia		2.63 ± 0.67	12.28 ± 1.92
ZBT	50	1.70 ± 0.81**	10.30 ± 2.28*
JSX	100	2.24 ± 0.45	11.34 ± 1.83
	200	1.90 ± 0.69*	10.45 ± 1.91*
	400	1.75 ± 0.37**	10.39 ± 2.41*

Discussion

Burnt hawthorn, malt, and medicated leaven are edible CMM. According to the theory of traditional Chinese medicine (TCM), they are channel tropism of the spleen, stomach, and liver, and have the functions

of promoting peristalsis of stomach and intestines. Edible CMM has little toxic effect on human body and is widely accepted. So the development and utilization of the source CMM have important social and economic benefits in the direction of the theory of TCM.

Some reports have previously shown that hawthorn has the lipid-decreasing effects.

No attention is paid to the study on the lipid-decreasing effects of the compound JSX. The characteristics of CMM is the synergistic effect among drugs. In the present study, we investigate the effects of JSX on reducing blood lipid. The findings are that JSX could significantly decrease the blood lipid in mice. The lipid-decreasing effects have no statistical difference between the high-dose JSX and ZBT groups.

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