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Effect of crude-herb moxibustion on blood lipids in rats with dyslipidemia

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KEYWORDS
High-lipid diet;
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Abstract *Objective:* To evaluate the lipid-regulating effect of crude-herb moxibustion on rats with dyslipidemia.
Methods: Fifty-four Sprague–Dawley rats were randomly divided into six groups with nine rats each. Control group rats were fed a normal diet, and bilateral acu-point Fenglong (equivalent to ST40 in humans) on the hind legs were covered with a placebo (general mucilage) for 2 hours each day. Model group rats were fed a high-lipid diet for 2 weeks. Therapy group rats were fed a high-lipid diet for 2 weeks and then administered crude-herb moxibustion at ST40 for 2 hours each day for the next 2 weeks. Prevention group rats were administered crude-herb moxibustion 2 hours a day for 2 weeks and then fed a high-lipid diet for the subsequent 2 weeks. Prevention/Therapy group rats were each administered crude-herb moxibustion at ST40 for 2 hours each day for 2 weeks, followed by a high-lipid diet for the next 2 weeks, and then crude-herb moxibustion again at ST40 for another 2 weeks. Simvastatin group rats were fed a high-lipid diet for 2 weeks and then treated with simvastatin for the next 2 weeks. Blood lipids, hepatosomatic indices (HSIs) and epididymal fat pad weights of all rats were examined.
Results: Compared with the Model group, levels of total cholesterol (TC), glycerinate, low-density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C) in plasma collected from the Therapy group, the Prevention/Therapy group, and the Simvastatin group were decreased. Moreover, compared with the Model group, HSIs in Therapy group rats were also decreased following administration of crude-herb moxibustion, but TC, TG, HDL-C, LDL-C, and VLDL-C levels in the Prevention group were higher than those in the Model group.

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Conclusion: The results reveal that blood lipids and HSIs appear to be modulated by the effect of crude-herb moxibustion and suggest therapeutic strategies for the treatment of dyslipidemia.

Introduction

Dyslipidemia is a metabolic disorder characterized by elevated levels of serum lipoproteins [1]. Dyslipidemia is one of the most important risk factors for coronary heart disease (CHD) and has been identified as the leading cause of death worldwide, causing global social and economic burden [2,3]. CHD is associated with obesity, type 2 diabetes mellitus, hypertension, stroke, and especially atherosclerosis [4,5]. The overall pooled prevalence of dyslipidemia in Chinese adults was estimated to be 41.9%, with a higher rate in men than in women [6].

Common drugs for dyslipidemia include niacin, folic acid derivatives, bile acid-binding resins, and 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitors, which are commonly known as statins. As one of the most widely used statin, simvastatin is highly selective for liver functions, inhibits synthesis of endogenous cholesterol, thus decreasing blood lipids. Simvastatin is a typical lipid lowering drug [7]. In numerous placebo-controlled clinical trials, statins have been shown to have well-defined safety profiles, but their side effects include liver damage and a small risk of muscle problems, such as myalgia, mild myositis, creatine kinase (CK) elevations, and rhabdomyolysis [8]. Previous clinical research indicates that mild myositis appears most often in controlled trials at an estimated rate of 5%–10% [9].

In traditional Chinese medicine (TCM), description of symptoms of the condition similar to dyslipidemia is found in the ancient text, *Yellow Emperor's Inner Classic*. In TCM, dyslipidemia is caused by a syndrome known as phlegm obstruction [10]. The acupoint Fenglong(ST40) is typically used to treat phlegm obstruction [11]. Accumulating evidence shows that acupuncture applied to ST40 has preventive and therapeutic effect on dyslipidemia, cardiovascular diseases, obesity, type 2 diabetes, hypertension, and stroke [12]. Electro-acupuncture (EA) or moxibustion applied to ST40 decreases plasma total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C), and increases high-density lipoprotein cholesterol (HDL-C) levels in dyslipidemic rats [13]. Acupuncture at ST40 also promotes hepatic gene expression and thus accelerates reversal of cholesterol transport and alleviates fat accumulation inside hepatocytes [14]. Additionally, acupuncture at ST40 in the rat model of hyperlipidemia lowers serum malondialdehyde (MDA) and endothelin-1 (ET-1) levels and improves superoxide dismutase (SOD) activity and nitric oxide (NO) and calcitonin gene-related peptide (CGRP) contents, and mitigates lipid peroxidation, clears free radicals [15,16]. Studies in the mice model of hyperlipidemia showed that acupuncture at ST40 regulates and ameliorates the metabolic balance of free radicals, and protects vascular endothelia [17,18]. At the gene level, research has shown that EA at ST40 in rats may have therapeutic effect on dyslipidemia that are mediated by increasing the expressions of the ABCA1, PPARα, LXR-α, and RXR-α mRNA, thus promoting the reversal of cholesterol transport [19].

Traditional Chinese herbal medicines influence multiple mechanisms in the human body. With regard to dyslipidemia, the herb hawthorn fruit (*Crataegus pinnatifida* Bge. var. major N. E. Br.) has been found to significantly decrease serum triglyceride (TG), TC, and LDL-C, and to reduce scores of pathologic changes due to hyperlipidemia while increasing HDL-C level [20,21].

Another TCM modality is crude-herb moxibustion, one of moxibustion therapies. Crude-herb moxibustion is application applied to the acupoint or the affected part with the herb of a constant irritant to skin till it presents red color, wistful or intravenous induction. Crude-herb moxibustion involves the synergistic effects of Chinese herbs and local acupoints.

Clinical research has found that crude-herb moxibustion is efficacious in the treatment of dyslipidemia. Crude-herb moxibustion applied to dyslipidemic rats can regulate general and liver fat metabolism, reduce fat deposits in the liver, inhibit fatty degeneration of liver cells, and restore normal liver cell function [22]. However, this report did find that crude-herb moxibustion exacerbates dyslipidemia [28]. Thus, more evidence is needed to validate the effects of crude-herb moxibustion on dyslipidemia.

This study investigated the therapeutic and preventive effects of the application of crude-herb moxibustion to acupoint ST40 in rats with dyslipidemia.

Materials and methods

Experimental animals

Fifty-four Sprague–Dawley rats (7 weeks old, 180 g–220 g) were acquired from Vital River Laboratories (Beijing, China) and housed in stainless steel cages in a controlled environment at 24°C±1°C and 70% relative humidity and maintained on a 12 hours light–dark cycle for at least 6 days prior to the experiments. The protocol was approved by Animal Care and Use Review Committee of Beijing University of Chinese Medicine and was in accord with the ethics guidelines of Beijing University of Chinese Medicine.

Preparations prior to crude-herb moxibustion

At the age of 8 weeks and after 1 week of acclimatizing to laboratory conditions, the rats were randomly assigned to either a control or a high-lipid diet (n = 9 rats/group). Body weight and food intake were recorded every 24 hours. The rats remained on their respective diets for 2 weeks. After fasting for 12 hours overnight, the rats were weighed.

Experimental animal grouping

Control group rats were fed a normal rat chow diet, and the acupoint ST40 of each hind leg was covered for 2 hours each day with a placebo consisting of general mucilage. Model group rats were fed a high-lipid rat chow diet for 2 weeks. Therapy group rats were fed a high-lipid diet for 2 weeks and then administered crude-herb moxibustion at bilateral ST 40 for 2 hours a day for the following 2 weeks. Prevention group rats were administered crude-herb moxibustion for 2 hours at bilateral ST40 each day for 2 weeks and then fed high-lipid diet for the following 2 weeks. Prevention/Therapy group rats were administered crude-herb moxibustion at bilateral ST40 for 2 hours each day for 2 weeks and then fed a high-lipid diet for the following 2 weeks, and subsequently underwent crude-herb moxibustion for 2 weeks. Simvastatin group rats were fed a high-lipid diet for 2 weeks and then treated with simvastatin at a dose of 4 mg/kg/d for an additional 2 weeks. Body weights of all rats were recorded daily.

Crude-herb moxibustion treatment

Prescription and preparation of the crude-herb moxibustion paste

The crude-herb prescription used in this study was referenced from *Chinese Acupoint Application Therapy* [23]. Herbs in the prescription were evodia fruit (*Evodia rutaecarpa* (Juss.) Benth. (Rutaceae)), 100 g; cuttle fish bone (*Sepia esculenta* Hoyle), 100 g; notoginseng root (*Panax notoginseng* (Burk.) F. H. Chen (Araliaceae)), 30 g; dragon's blood resin (*Daemonorops draco* Bl. (Palmae)), 30 g; chicken gizzard lining (of *Gallus gallus domesticus*), 50 g; processed pinellia rhizome (*Pinellia ternate* (Thunb.) Breit.), 50 g; aged tangerine peel (*Citrus reticulata* Blanco), 20 g; turmeric rhizome (*Curcuma longa* L. (Zingiberaceae)), 15 g; hawthorn fruit (*Crataegus pinnatifida* Bge. var. major N. E. Br.), 30 g; and white mustard seed (*Sinapis alba* L.), 100 g. All of the ingredients with the exceptions of dragon's blood powder and notoginseng root powder were diluted 10-fold in water and boiled twice for 1.5 hours each time to create a concentrated liquid extract. The water and oil phases of the substrate were placed in a beaker and a conical flask with plug, respectively, and heated in water bath at 80°C. The liquid extract, powders of Dragon's blood and Notoginseng root were set in the water phase and mixed. The water phase was poured into the oil phase and then mixed again for 5 minutes at 80°C. The compound was removed, milled, and refrigerated in the mortar. The liquid extract weighed 440 g. The substrate was composed of albolene (40 g), glycerin monostearate (10 g), adeps lanae (10 g), Tween-80 (48 g), and Span-60 (12 g), glycerin (40 g). The crude-herb liquid extract and substrate were mixed together to make a paste. The paste was processed by the Chinese Pharmacy School of Beijing University of Chinese Medicine.

Selection and stimulation of acupoints

Based on the animal acupoint reference book *Experimental Acupuncture Science* [24], ST40 in the rat is at the midpoint of the posterior margin of the fibula (Fig. 1).

Method of crude-herb moxibustion

The day before treatment, an area of approximately 1 cm over the surface of ST40 was depilated. On the day of treatment, the rats were bagged and their hind legs were exposed outside of the bag. The herbal paste was mixed with minced ginger (3 g) and garlic (3 g) and smeared on ST40 over an area of approximately 0.7 cm. Two hours later, the paste was washed off with water. The sites exhibited some redness but no blisters.

Test methods

Procedure

All rats were fasted for 12 hours overnight, weighed, and then anesthetized with urethane dissolved in distilled water and injected intraperitoneally (i.p.) at a dose of 4 mg/kg. Anesthesia was deemed adequately when pedal reflexes were no longer present. The rats were placed on a small platform and their limbs secured. The gross anatomy was observed, and 1.5 mL of blood was drawn from the abdominal aorta and placed in a Vacutainer blood collection tube. The blood samples were centrifuged at 2000 g for 10 minutes at 4°C. The plasma was then collected to determine levels of TC, TG, LDL-C, and HDL-C. The entire liver and one side of the epididymal fat pad were rapidly dissected out, and their weights recorded. The samples were then stored at −80°C.

Hepatosomatic index and epididymal fat pad weight

Hepatosomatic index (HIS) is the ratio of liver weight to body weight [25]. After adaptive feeding, the rats were weighed. Before anesthesia, the rats were weighed again. The weight was measured twice, and its change obtained. Epididymal fat pad weight was taken as the weight of one side of the epididymal fat pad [26,27].

TC, TG, LDL-C, and HDL-C levels

Standard kits from Beckman Ltd. (Fullerton, CA, USA) were used for the quantitative determination of plasma triglycerides, cholesterol, HDL-C, LDL-C, and VLDL-C, which were measured with an auto analyzer (Beckman Instruments 5821, Fullerton, CA, USA) using their respective standards. Triglycerides and cholesterol were used with the peroxidase method, and HDL-C, LDL-C, VLDL-C were assessed using the direct method.

Statistical analyses

The analyses were performed with the SPSS 18.0 statistical software. Data are presented as the means ± the SEMs. The groups were compared with one-way ANOVAs and Tukey's tests. *P* values below 0.05 were considered significant.

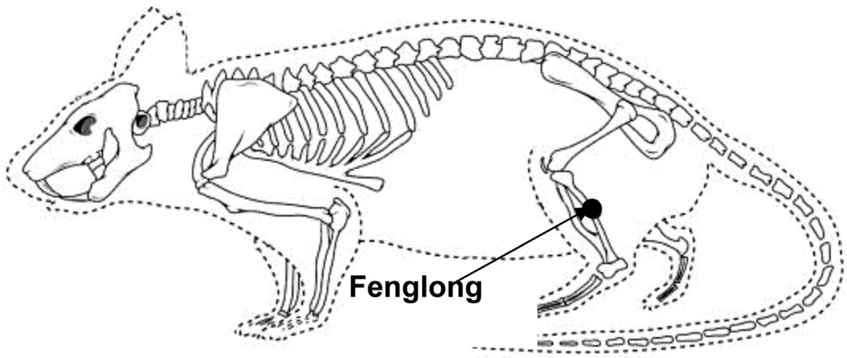

Figure 1 Location of ST40 in the rat.

Results

Effect of crude-herb moxibustion on TC, TG, LDL-C, and HDL-C

Total cholesterol level in the Model group was significantly higher (by seven fold) than that in the Control group ($P < 0.05$) (Fig. 2). TC levels were significantly lower in the Therapy group and in the Prevention/Therapy and Simvastatin group compared with the Model group ($P = 0.000$, $P = 0.038$, $P = 0.000$, and $P = 0.039$, respectively). Surprisingly, compared with the Model group, TC level in the Prevention group was elevated ($P = 0.025$). Moreover, TC levels in the Therapy group and Prevention/Therapy group were similar to that of the Simvastatin group (Fig. 2).

Triglyceride level in the Model group was significantly different from that of the Control group ($P = 0.038$). Compared with the Model group, TG levels in the Therapy group and in the Prevention group were elevated, but these differences were not significant ($P = 0.249$). TG level in the Prevention/Therapy group was lower than that of the Model group and similar to that of the Simvastatin group (Fig. 3).

HDL-C level in the Model group was markedly increased compared to that of the Control group ($P = 0.000$) (Fig. 4). Compared with the Model group, HDL-C level in the Prevention group was elevated ($P = 0.074$), but HDL-C levels in the Therapy group, Prevention/Therapy group, and Simvastatin group were decreased compared to that of the Model group (Fig. 4).

The LDL-C level in the Model group was 15-fold greater than that of the Control group ($P = 0.000$) (Fig. 5). However, levels of LDL-C in the Therapy group and Prevention/Therapy and Simvastatin group were extremely reduced compared with that of the Model group (Fig. 5).

VLDL-C level in the Model group was significantly different from that of the Control group ($P = 0.039$) (Fig. 6). VLDL-C level in the Prevention group was higher than that of the Model group, but this difference was not significant ($P = 0.254$). Compared with the Model group, levels of VLDL-C in the Therapy group were elevated, but level in the Prevention/Therapy group was lower, although these differences were not significant ($P = 0.249$) (Fig. 6).

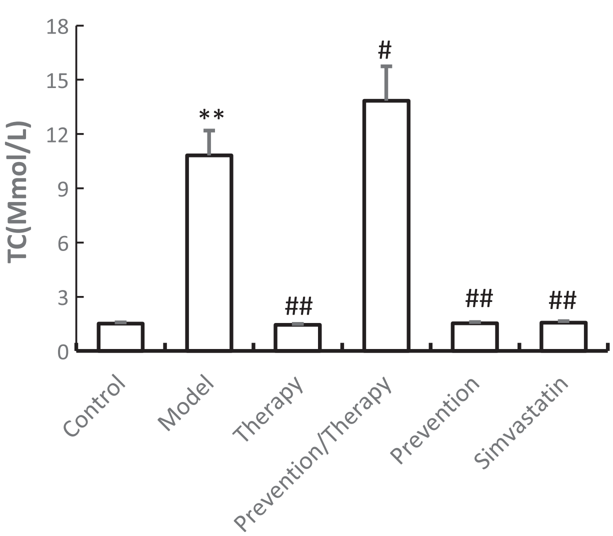

Figure 2 Total cholesterol levels among different rat groups. **: $P < 0.01$ significantly different from Control group. #: $P < 0.05$, ##: $P < 0.01$ significantly different from Model group.

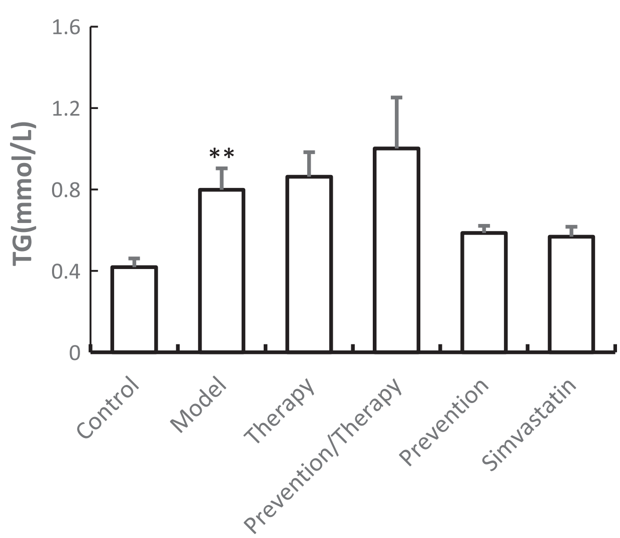

Figure 3 Triglyceride levels among different rat groups. **: $P < 0.01$ significantly different from Control group.

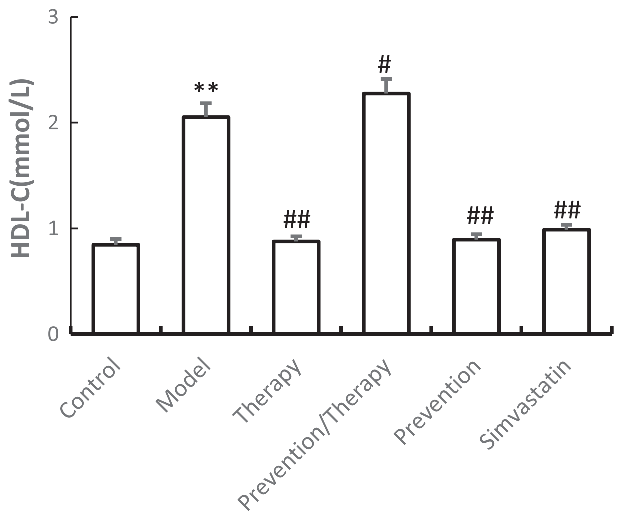

Figure 4 HDL-C levels among different rat groups. **: $P < 0.01$ significantly different from Control group; #: $P < 0.05$, ##: $P < 0.01$ significantly different from Model group.

Effect of crude-herb moxibustion on HSI, weight change, and epididymal fat pad weight

There was a significant difference between the rats in the Control group and those in the Model group ($P = 0.000$) (Fig. 7). Levels of HSI in the Therapy group, Prevention/Therapy group, and the Simvastatin group were significantly decreased compared to that of the Model group ($P = 0.000$, $P = 0.000$, $P = 0.001$, and $P = 0.000$, respectively). Body weight in the Model group were slightly reduced compared to that of the Control group. Rats in the Prevention/Therapy group were the heaviest of all groups. Epididymal fat pad weight was similar across all groups.

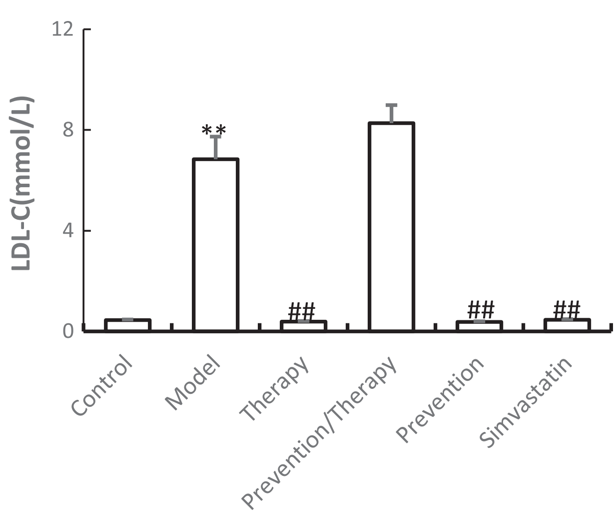

Figure 5 LDL-C levels among different rat groups. **: $P < 0.01$ significantly different from Control group; #: $P < 0.05$, ##: $P < 0.01$ significantly different from Model group.

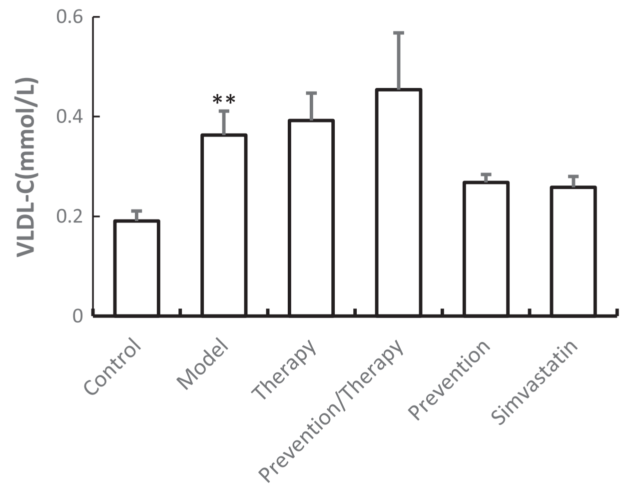

Figure 6 VLDL-C levels among different rat groups. **: $P < 0.01$ significantly different from Control group.

In summary, the levels of TC, TG, LDL-C, and VLDL-C in the Therapy group, Prevention group, Prevention/Therapy group and Simvastatin group were significantly decreased, whereas level of HDL-C was decreased compared with that of the Model group.

Discussion

Blood lipids, weight, and HSI

Our study revealed that crude-herb moxibustion administered at acupoint ST40 prevented and treated dyslipidemia in rats caused by high-lipid diet. Specifically, crude-herb moxibustion appears to exert a regulatory effect on levels of TG, HDL-C, and LDL-C. Additionally, crude-herb moxibustion decreased levels of TC, LDL-C, and HSI and slightly elevated VLDL-C. These results show that crude-herb moxibustion might have preventive and therapeutic effect against dyslipidemia and thus atherosclerosis.

In this study, a rat model of dyslipidemia was successfully implemented with a high-lipid diet over 2 weeks. The levels of blood lipids in the Therapy group, Prevention/Therapy group, and Simvastatin group exhibited notable decreases compared with the Model group. These results may indicate that crude-herb moxibustion causes a decrease in blood lipids, though rats in the Prevention group exhibited higher blood lipid levels than rats in the Model group. Thus, when applied preventively, crude-herb moxibustion might be associated with a risk of elevated blood lipids. More research is needed for explanation. Interestingly however, the effect of moxibustion observed in the Prevention/Therapy group was better than that observed in the Therapy group. It probably benefits from more quantity of stimulus. These findings indicate that prevention and therapy with crude-herb moxibustion had a more positive effect than crude-herb moxibustion alone in rats with dyslipidemia but the preventive effect of crude-herb moxibustion require further exploration.

Crude-herb moxibustion and Simvastatin had therapeutic effect on HSI. Dyslipidemia results in liver weight gain,

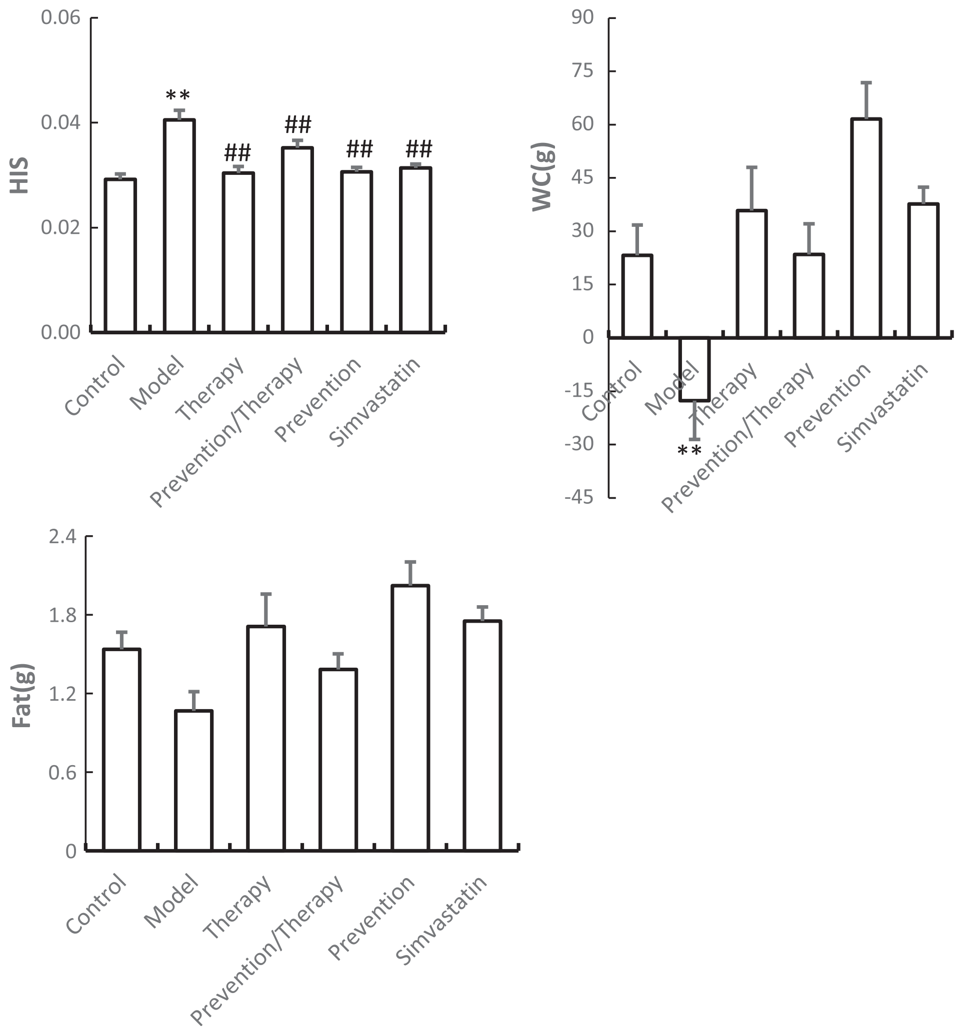

Figure 7 Comparison of the HIS, weight change and epididymal fat weight levels among different rat groups. **:P $<$ 0.01 significantly different from Control group; ##: P $<$ 0.05 significantly different from Model group.

which is primarily exhibited via HSI. Thus, in this study, HSI was markedly elevated in dyslipidemic rats compared with normal rats. Crude-herb moxibustion significantly decreased HSI in SD mice compared with that of the Model group, hence preventing liver weight gain, which indicates that such treatment might protect the liver from blood lipid accumulation.

Body weight decrease indicated that dyslipidemia does not always induce weight gain and weight gain does not always coincide with a rise in blood lipids. Although the body mass index is positively correlated with the risk of dyslipidemia and is clinically predictive of dyslipidemia [28], there is evidence that changes in blood fat are unrelated to weight [29]. One likely explanation is that rats in the Model group were not accustomed to the high-lipid diet and reduced food intake. However, the blood lipid disorders in the rats resulted from damage due to the intake of high-lipid food, which did not lead to obesity in the short-term. Additionally, although high-lipid diet led to a decrease in body weight, the impact of the diet was less than that of natural growth. Thus, the body weight of the Prevention/Therapy group increased over a longer period, and the high-lipid diet was withdrawn at a later time.

The epididymal fat pad weight was not different among the groups. Ineffectiveness might result from the fact that the short-term high-lipid diet did not bring about changes in body fat.

Results of our study are in accord with those of previous research regarding crude-herb moxibustion [30]. Our results revealed that crude-herb moxibustion seemed regulate general and liver fat metabolism.

Comparative analysis of crude-herb moxibustion, preventive crude-herb moxibustion, and simvastatin

Compared with the Model group, TG level in the Prevention/Therapy group was slightly reduced; however, other measures (i.e. TC, HDL-C, LDL-C, VLDL-C, HSI, body weight, and epididymal fat pad weight) did not exhibit differences. Thus, preventive crude-herb moxibustion did not show good effect on rats with dyslipidemia. The probable reason for this finding is that preventive treatments for dyslipidemia need to be individualized. In TCM, doctors focus on syndrome differentiation and provide targeted therapy to transform the patient's condition from dysharmony to harmony. Moreover, increasing evidence suggests that crude-herb moxibustions applied after syndrome differentiation has superior effect [31].

However, the mechanisms of the effect of crude-herb moxibustion on dyslipidemia remain unknown. After 2 weeks of therapy, rats in the Therapy group and the Simvastatin group exhibited blood lipid levels that were similar to that of the Control group. These findings indicate that dyslipidemia can be regulated with therapy and that crude-herb moxibustion had positive effect. Compared with the Simvastatin group, the Crude-herb moxibustion group exhibited better results in terms of LDL-C level but worse results in terms of TC, TG, HDL-C, and VLDL-C levels.

The present study has limitation. The process of dyslipidemia model might be reversible. This study found that rats recovered from dyslipidemia beginning on Day 12 of the high-lipid diet. However, there is longer feed time in the Prevention/Therapy group, so this group has less reliability.

Conclusions

This study revealed the therapeutic effect of crude-herb moxibustion on rats with dyslipidemia. Future studies are needed to elucidate the mechanism of crude-herb moxibustion and how individual ingredients contribute to the combined pharmacologic efficacy. Moreover, the question raised in this study regarding tendency toward body weight changes and weight changes of the epididymal fat pads are also to be explored in future research.

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