殖有一定的刺激作用, 但随着苦参碱质量浓度的增高, 这种协同刺激作用又随之下降。此结果提示苦参碱不能促进静止的小鼠 PBL 的增殖反应, 对 ConA 活化了的小鼠脾细胞增殖, 高质量浓度的苦参碱也有一定的抑制作用, 显示了其具有免疫抑制剂的特征。苦参碱对静止和活化的小鼠 PBL 体外增殖活性的不同影响, 也可能与机体的免疫状态有关, 本实验中所制备的 PBL 来自荷瘤小鼠, 受肿瘤影响, 小鼠 PBL 对刺激原的反应性与正常情况可能有所不同。苦参碱对荷瘤机体免疫效应细胞活性的影响和具体的作用机制还有待于深入研究。

本研究中, 苦参碱作用后小鼠的胸腺外观与对照组几乎无差异, 胸腺指数无明显改变, 而脾指数明显低于对照组, 以高剂量苦参碱作用更甚, 表明苦参碱在一定程度上抑制了荷瘤小鼠的免疫功能, 其中对脾脏的抑制作用强于胸腺。病理形态学观察也证明了这一点。

在机体的抗肿瘤免疫反应中, IL-2 和 IL-12 是发生细胞免疫应答所必需的两种细胞因子, 通过多种途径发挥免疫调节作用。本研究中, 苦参碱治疗后小鼠血清 IL-2 和 IL-12 水平没有增高, 提示苦参碱不能增强荷瘤小鼠的抗肿瘤细胞免疫应答反应。但也不能排除肿瘤发生时, 机体所产生的免疫抑制因子的作用。

本研究发现, 苦参碱对肿瘤所致的小鼠免疫功能低下状态没有明显改善作用, 同时体内、体外实验都显示其有一定的免疫抑制效应, 表明苦参碱的抑瘤活性不是通过提高机体自身的免疫机能来实现的, 还存在其他的作用机制, 其确切的抗肿瘤作用尚待深入研究。

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Weight-reduction and fat-decrease effects in rats of extracts from five kinds of Chinese herbs

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Abstract: Object To evaluate the effects of the extracts from five kinds of Chinese herbs (5ECH) on weight-reduction and fat-decrease in rats, as well as their mechanisms. **Methods** Rats were fed by high-calorie food and administrated with two different doses of 5ECH as experimental groups, with Qumei and Xuezhikang as positive control groups, and with carboxymethyl cellulose sodium as negative control group by ig administration with relevant drugs in each groups. Concentrations of triglycerides (TG) and total cholesterol (TC) in the blood were determined by a HITACHI—7170A auto-biochemical analyzer. The activities of fatty acid synthase (FAS) and acyl-coenzyme A synthetase (ACS) were determined by spectrophotometry and isotopic-labeling, respectively. The items of body weight increase (BWI), body fat (BF), and food intake (FI) were determined by regular weighing. **Results** 5ECH reduces BWI, BF, TG, TC, and FI in a dose-dependent manner, inhibits FAS activity, and enhances ACS activity in a dose-dependent manner. The BWI is highly correlated to the FI; the TG and TC levels in the blood are highly correlated to the activities of FAS and ACS, and the BF is highly correlated to both the FI and the two enzymes' activities. **Conclusion** The 5ECH in this research has significant weight-reduction and fat-decerase effects on rats. The effects are associated with appetite suppression, FAS inhibition, and ACS

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activation.

Key words: obesity; hyperlipimedia; weight-reduction; fat-decrease; Chinese herbs; fatty acid synthase (FAS); acyl-coenzyme A synthetase (ACS)

5 种中草药提取物对大鼠的减肥降脂作用

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摘 要: **目的** 检验 5 种中草药提取物 (5ECH) 对大鼠的减肥降脂作用及其机制。**方法** 喂饲高脂饲料制备肥胖高脂大鼠模型, 5ECH 各设两个剂量为实验组, 曲美和血脂康为阳性对照组, 羧甲基纤维素钠为阴性对照组, 各组均 ig 相应药物。TACHI-7170A 自动生化分析仪测定血浆甘油三酯 (TG) 和总胆固醇 (TC) 含量; 分光光度法和同位素法测定脂肪酸合成酶 (FAS) 及脂酰辅酶 A 合酶 (ACS) 活性。常规称重法测定各组大鼠体重增长指数 (BWI)、脂肪质量 (BF) 及摄食量 (FI)。**结果** 5ECH 对大鼠 BWI、BF、TG、TC 和 FI 具有剂量依赖性抑制作用; 并剂量依赖性地抑制 FAS 活性、提高 ACS 活性。BWI 与 FI 具有高度相关性, TG 和 TC 水平与 FAS 及 ACS 具有高度相关性, BF 与 FI 及两种酶的活性具有高度相关性。**结论** 5ECH 通过抑制食欲、抑制 FAS 活性和促进 ACS 活性而对高脂饮食诱导的肥胖大鼠具有显著的剂量依赖性减肥降脂作用。

关键词: 肥胖; 高脂血症; 减肥; 降脂; 中草药; 脂肪酸合成酶; 脂酰辅酶 A 合酶

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Obesity is seriously threatening human health. Many clinical trials have been made to control obesity by depressing energy intake. Sibutramine and Orlistat are two drugs currently available to control obesity, suppress appetite, and block fat absorption through the small intestine, respectively[1-4].

Fatty acid synthase (FAS, EC 2.3.1.85) and acyl-coenzyme A synthetase (ACS, EC 6.2.1.3) are two key enzymes in fat synthesis and fat degradation-metabolism *in vivo*, respectively. It is believed that substances acting on one end of fat metabolic pathway may fail to achieve significant long-term efficacy in the control of body weight and body fat, because of the decreases in fat synthesis or increases in fat degradation result by compensatory adjustments. From this point of view, FAS and ACS, the two key enzymes in lipid metabolism, may be very effective when used together as screening targets for natural products that can promote weight-reduction and fat-decrease.

FAS and ACS were previously used as *in vitro* targets to screen 40 Chinese herbs widely used to treat different types of obesity and hyperlipidemia[5-8]. Three Chinese herbs' extracts were found to inhibit FAS activity, while two enhanced ACS activity. Specifically, the water-extracts from cassia seeds (*Semen Cassiae*, the dried ripe seed of *Cassia obtusifolia* L., Leguminosae), Oolong tea [*Camellia Sinensis*, the dried buds and leaves of *Camellia sinensis* (L.) O. Ktze, Theaceae], and chrysanthemum [*Flos Dendranthemate*, the dried flower of *Dendranthema morifolium* (Ramat.) Tzvel., Compositae] strongly inhibit FAS activity *in vitro*. Water-extracts from tangerine peel (*Pericarpium Citri Reticulatae*, the dried peel of *Citrus reticulata* Blanco, Rutaceae), and corn silk (*Stigma Maydis*, the dried stigma of *Zea mays* L., Gramineae) significantly increase ACS activity *in vitro*. In the current studies, the water-extracts from these five Chinese herbs (5ECH) were combined and injected intragastrically in rats to evaluate the extracts' weight-reduction and fat-decrease effects and to examine the appetite suppression, FAS inhibition, and ACS activation associated with these effects.

1 Experiment

1.1 Materials and animals

1.1.1 Chinese herb materials: The five kinds of Chinese herbs were collected from Anguo Chinese Medicine Market in Hebei Province in 2001 and identified by Prof. WANG Zhao, from Institute of Traditional Chinese Medicine, College of Life Science and Technology, Tsinghua University.

1.1.2 A drug for obesity produced by Taiji En-

terprise Group (China) with Sibutrimine as its bioactive component (Qumei).

1.1.3 A drug for hyperlipidemia produced by Beidaweixin Company (China) with Lovastatin as its bioactive component (Xuezhikang).

1.1.4 Subjects and feeding conditions: Forty male Spraque-Dawley rats, aged from 3—4 weeks, were purchased from the Experimental Animal Center of the Academy of Military Medical Science, Beijing, China. The rats were randomly placed into five groups. Each rat was raised in a single cage numbered from 1 to 8 in each group. Food and water for all rats supplied at will.

1.1.5 Diet prescription: The food for the rats is prescribed according to "The Procedures and Methods for Health Food Evaluation" issued by the Health Supervision Department of the Health Ministry of the People's Republic of China[9].

1.2 Methods

1.2.1 Preparation of 5ECH and the suspensions: The proportions of the five Chinese herbs and the preparation of 5ECH were determined according to the effectiveness of the water-extracts of the five Chinese herbs in inhibiting FAS and activating ACS activities[5,6]. Specifically, to mince separately 300 g cassia seeds, 180 g Oolong tea, 150 g chrysanthemum, 150 g tangerine peel, and 120 g corn silk; to dim separately the five materials in two-time volume of distilled water of 20—30 ℃ for 2 hours, then cook at 90—100 ℃ for 30 minutes; to lay up and let cool, then combine the five together; to filtrate with eight layers of gauze, then to centrifuge the filtrates at 1 000×g for 15 minutes, and collect the supernatant. The pH of the supernatant should be ranged from 7.2 to 7.4, and soluble solids in it should be ranged from 80 to 90 mg/mL. Making the supernatant freeze dried, about 120 g powder was obtained. The powder should appear light yellow and completely water-soluble. 5ECH, Qumei, and Xuezhikang were all made into suspensions according to the required doses with car-boxymethyl cellulose sodium of 0.8%.

1.2.2 Doses and administrations: 5ECH (0.5, 3.0 g/kg), Qumei (Sibutrimine 7.2 mg/kg), Xuezhikang (Lovastatin 14.4 mg/kg), and carboxymethyl cellulose sodium were administrated to rats intragastically once a day. The feeding lasted for five weeks. The administration volume for each time in all five groups was 1.0 mL per rat.

1.2.3 Isolation, purification, and activity measurements of FAS and ACS: Three of the eight rats in each group were chosen for the determination of FAS and ACS activities. The isolation, purification, and activity measurements of FAS from rat fat tissue followed the protocols described by Takao T and Kohei H[10]. The isolation, purification, and activity measurements of ACS from rat liver followed the protocols described by Fazal A and Patricia A[11]. Protein was quantitatively determined by the methods of Smith P K[12] using bovine serum albumin as a standard.

1.2.4 Blood sampling, rat killing, and fat collection: The blood of each rat was sampled from the reto-orbital sinus of the eyes. The rats were sacrificed following blood sampling by decapitation after anesthesia. The subcutaneous, perirenal, and epididymal fat pads of each rat were carefully and completely peeled, collected, and weighed.

1.2.5 TG and TC tests: Concentrations of TG and TC in the blood were determined by a HITACHI—7170A auto-biochemical analyzer.

1.2.6 Data statistics: Data for all the tests were expressed as a mean value ± standard deviation ($\bar{x} \pm s$). One-way ANOVA was used for comparison between the groups. Significant difference was accepted at values of $P < 0.05$. Linear regression was used for correlation coefficient (r) determination.

2 Results

2.1 Effects of 5ECH on the body weight increase (BWI): The 5ECH inhibits the BWI significantly ($P < 0.05$, refer to Table 1). 5ECH, both at high dose and at low dose could reduce the BWI by 9% and 7%, respectively, indicating that 5ECH's weight-reduction effect is dose-dependent. Qumei reduces the BWI by 22% compared to that of the control. These results show that, although not as

effective as Qumei, 5ECH has a significant effect in reducing BW I

2.2 Effects of 5ECH on body fat (BF): 5ECH decreases BF significantly ($P < 0.05$, refer to Table 1). 5ECH at high dose, 5ECH at low dose, Qumei, and Xuezhikang decrease BF by 28%, 16%, 23%, and 8%, respectively, compared to that of the control. This demonstrates that 5ECH has a significant fat-decrease effect, and is more effective than Qumei and Xuezhikang at its high dose. The difference in the decrease of BF between 5ECH at low dose and 5ECH at high dose is significant ($P < 0.05$, refer to Table 1), indicating that 5ECH's fat-decrease effect is dose-dependent.

2.3 Effects of 5ECH on the concentrations of TG and TC in the blood: 5ECH decreases TG and TC concentrations in the blood significantly ($P < 0.05$, refer to Table 1). Compared to the control, 5ECH at high dose reduces TG and TC in the blood by 51% and 42%, respectively; 5ECH at low dose by 10% and 18%, respectively; Qumei by 7% and 5%; and Xuezhikang by 30% and 42%, respectively. The above results indicate that 5ECH has a significant inhibitory effect on the concentrations of TG and TC in the blood. Its effectiveness, as at high dose, is similar to that of Xuezhikang. The differences in TG and TC between 5ECH both at low dose and at high dose are significant ($P < 0.05$, refer to Table 1), indicating that 5ECH has a dose-dependent effect in reducing blood TG and TC.

2.4 Effects of 5ECH on food intake (FI): 5ECH inhibits FI significantly ($P < 0.05$, refer to Table 1). Compared to that of the control, 5ECH at high dose, 5ECH at low dose, and Qumei reduce FI by 11%, 8% and 18%, respectively. The above data suggest that 5ECH has a significant inhibitory effect on appetite, although the effect is not as efficient as Qumei. The difference in FI between the high dose and low dose of 5ECH is significant ($P < 0.05$, refer to Table 1), indicating 5ECH's effect of depressing appetite is dose-dependent.

2.5 Effects of 5ECH on activities of FAS and ACS *in vivo*: 5ECH inhibits FAS activity and enhances ACS activity ($P < 0.05$, refer to Table 1). 5ECH at high dose, 5ECH at low dose, Qumei, and Xuezhikang inhibit *in vivo* FAS activity by 50%, 24%, 9%, and 6%, respectively, and enhance *in vivo* ACS activity by 146%, 42%, 6%, and 22%, respectively. The above results reveal that 5ECH is more effective in inhibiting FAS activity and in enhancing ACS activity than Qumei and Xuezhikang. The differences in the FAS and ACS activities between the high dose and low dose of 5ECH are significant ($P < 0.05$, refer to Table 1), indicating that 5ECH's inhibition of FAS and activation of ACS *in vivo* are dose-dependent.

Table 1 Effects of 5ECH on BW I, BF, FI, concentrations of TG and TC in blood, and activities of FAS and ACS in vivo

Groups	Dose /(g·kg^{-1})	SBW /g	BW I /g	BF /g	TG /(μmol·L^{-1})	TC /(μmol·L^{-1})	FI /g	FAS/(Kat, ×10^9, means)	ACS/(Kat, ×10^9, means)
Control	-	48.9±3.3	276.2±12.2	25.2±2.0	3.35±0.29	3.59±0.42	666.3± 31.3	232.3	395.0
5ECH	3.0	50.6±4.1	252.2±10.7$^{*\triangle}$	18.1±1.6$^{*\triangle}$	1.63±0.26$^{*\triangle}$	2.09±0.35$^{*\triangle}$	593.7± 31.4$^{*\triangle}$	117.3$^{*\triangle}$	969.7$^{*\triangle}$
	0.5	49.6±4.2	257.3±11.0*	21.1±1.1*	3.00±0.26*	2.96±0.37*	616.4± 38.2*	176.0*	561.7*
Qumei	-	49.2±4.1	216.8±16.5*	19.5±2.1*	3.13±0.44	3.40±0.37	547.4±108.5*	210.7	419.0
Xuezhikang	-	48.1±3.4	274.9±11.5	23.2±1.4	2.35±0.28*	2.09±0.34*	653.9± 52.0	218.0	483.3*

SBW - starting body weight. $\bar{x}\pm s$, $n = 8$ for SBW, BW I, BF, TG, TC, and FI; $n = 3$ for FAS and ACS. One-way ANOVA was used for comparison between groups; significant difference was accepted at values of $P < 0.05$; $^{*}P < 0.05$ *vs* that of control; $^{\triangle}P < 0.05$ *vs* that of 5ECH at low dose

2.6 Correlation coefficients (r) among the items: Among the five groups, the BW I is closely related to FI ($r_{FI/BW\,I} = 0.978$), suggesting that a rat's body weight is closely related to their appetite. The TG and TC are highly correlated to FAS ($r_{FAS/TG} = 0.978$ and $r_{FAS/TC} = 0.576$), and ACS ($r_{ACS/TG} = 0.904$ and $r_{ACS/TC} = 0.678$), showing that the fat and cholesterol levels in the blood are closely related to the activities of FAS and ACS *in vivo*. The BF is highly correlated to FI, FAS, and ACS ($r_{FI/BF} = 0.853$, $r_{FAS/BF} = 0.793$, $r_{ACS/BF} = 0.691$), indicating that the fat amount in the body is closely related to appetite and the metabolism.

3 Discussions

The results of the study demonstrate 5ECH has significant weight-reduction and fat-decrease effects on rats. These weight-reduction effects, although not as effective as Qumei, are significant while compared to that of the control. 5ECH reduces BF, the concentration of TG, and the concentration of TC in the blood. These fat-decrease effects are more effective than that of Xuezhikang. A conclusion could be reached from the above facts that in regard of weight-reduction and fat-decrease, 5ECH, with the two drugs as positive controls, has significant effects and is the potential of being developed into weight-reduction and fat-decrease drugs.

Many types of Chinese herbs have been used in the treatment of obesity and hyperlipidemia for thousands of years in China, but their mechanisms of action remain unknown. In this work, that Chinese herbs reduce BW I, BF, TG and TC levels in the blood through depressing appetite, inhibiting FAS activity, and activating ACS activity is demonstrated. These results support the previous studies that the activities of FAS and ACS might be relevant to the body weight and body fat[13, 14].

The research confirms the hypothesis that Chinese herbs' efficacy may be attributed to the diversity of chemical components that act on many targets simultaneously, because in the five herbs there are many kinds of chemical constituents, such as flavonoids, anthraquinones, flavanols, alkaloids, long chain fatty acid derivatives, terpenoids, and trace substances. The results indicate that multi-target effects may be especially important for anti-obesity and anti-hyperlipidemia drugs, for the substances that act on only one end of the fat metabolic pathway may fail to achieve significant long-term efficacy, for a decrease in fat synthesis or an increase in fat decomposition will result in compensatory adjustments.

Now, a cell-based assay system for high-throughput screening of the potential chemicals from Chinese herbs, and a vast resource of chemical constituents with anti-obesity and anti-hyperlipidemia activities are to be established. The effectiveness of Chinese herb extracts, such as 5ECH in weight-reduction and fat-decrease, could be increased and the clinical effective doses could be decreased with the active components having been screened and identified.

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