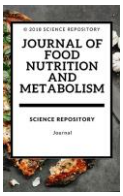

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Research Article

Effect Evaluation of Transfat Decoction on Obesity Mice Induced by High-Fat Diet

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ABSTRACT

Objective: To investigate the effect of Tranfat Decoction on obesity mice induced by high-fat diet. **Methods:** 90 healthy SPF C57BL/6 male mice aged 3 weeks were selected and 15 of them were treated as normal group. The remaining 75 mice were used as model group to induce obesity. After successful modeling, drug intervention was given for 6 weeks, and body weight, body fat content and blood fat level of the mice were measured.

Results: After 6 weeks of drug intervention, compared with the model blank group, the body weight of the drug administration group all decreased to different degrees *($P < 0.05$)*. TC, TG, LDL-C and HDL-C levels were significantly different in the high-dose group *($P < 0.05$)*. Fat volume was lower in the administration group *($P < 0.05$)*.

Conclusion: Tranfat Decoction can reduce body weight, body fat content and blood fat level in obese mice induced by high-fat diet.

Introduction

With the development of modern society and the increase of survival pressure, human nutrition patterns and lifestyles have undergone a landmark transformation, and the most direct result is the significant increase of the incidence of obesity. Data show that in 2014, there were 1.9 billion overweight adults in the world, among which more than 600 million were obese patients [1]. Obese patients are often accompanied by abnormal blood glucose and lipids, which are often high-risk groups for hypertension, diabetes and cardiovascular and cerebrovascular diseases. Meanwhile, obesity also has negative effects on reproductive health. Obesity has become a chronic disease that seriously endangers human life, and its treatment has become the focus of medical attention. Although western medicine has a quick effect in treating obesity, it has a large side effect on the digestive system and is easy to rebound. From the perspective of the overall concept of Chinese medicine, the side effect is small, and the effect is stable. Based on this situation, this project intends to carry out intervention treatment on obese mice induced by high-fat diet with Tranfat Decoction and evaluate the effect of Tranfat Decoction on obese mice induced by high-fat diet by detecting their body weight, body fat content and blood fat level.

Materials and methods

I Experimental animals

At 3 weeks of age, 90 SPF healthy C57BL / 6 male mice weighed about 10g. (Beijing weitonglihua experimental animal technology co., LTD., SCXK (Beijing) 2016-0006)

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II Experimental reagents and instruments

Reagent	**Company**
Anhydrous ethanol	Tianjin huadong reagent, China
Pentobarbital sodium	Beijing lantai chemical technology co. LTD
Instrument	Company
Electronic balance	China huangshi hengfeng medical equipment co. LTD
ST16R low-temperature centrifuge	Thermo scientific,inc
Automatic biochemical analyzer (7600-110)	Hitachi co., LTD
Quantum FX microCT	PerkinElmer

III composition and preparation of TCM

III - I composition and solution

Tranfat Decoction: Dried Rehmannia Glutinosa (25g), Cornus Officinalis (15g), Yam (15g), Poria (12g), Alisma (12g), Peony Bark (9g), Cinnamon (3g), Pueraria (25g), Ginseng (9g), Astragalus (15g), Rhizoma Coptidis (6g), Ginger (6g), Jujube (3g). The prescription is based on Shenqi Pill, which is made of Aconite, Ginseng and Rhizoma Coptidis. Kidney is water viscera, inside the Mingmen Fire. Kidney Yang is the Yuan Yang of body. Spleen Yang is also from the Kidney Yang, only enough Kidney Yang can make the Spleen Yang enough. Traditional Chinese Medicine calls fat as phlegm, so called "fat person has much phlegm".Only Spleen get strong can move normally to digest the food, clear phlegm and wet. This is the reason using Shenqi Pill aid Kidney Yang. Phlegm is Yin evil, this namely Wang Bing "benefit the source of fire, in order to eliminate Yin" reason. With Dried Rehmannia Glutinosa tonifying Kidney Yin, as King medicine. Cornus Officinalis and Yam tonifying liver, spleen and blood, as Minister medicine. With Poria tonifying spleen and drying dampness, Alisma regulating waterways, Peony Bark clearing and purging liver-fire as adjuvants, these three medicines contain purging and tonifying to make the pathogenic factors go away and make the tonifying effective and make the nourishing Yin medicines won't help the dampness. A little cinnamon as envoy, using Yang to make Yin tonified and not greasy, that is, "Good Yin, will be in the Yang. Good Yang will be in the Yin", and cinnamon has the power to make fire back to the source, weight loss without injury. Pueraria mainly enters the spleen and stomach meridian and has the ability to clear the spleen and stomach. Ginsenoside Rb1 is capable of upregulation of the expression of peripheral lipids in adipose tissue, reducing the release of free fatty acids and the heterotopic deposition of triglycerides [2]. Astragalus is a medicine for reinforcing Qi, which can replenish Sanjiao. Zhiyuan Zhang, an old doctor of traditional Chinese medicine, chose to use large dose of Astragalus to treat obesity, for obesity patients who lack of exercise, drowsiness, and loose stools. Rhizoma Coptidis mainly enters the heart meridian, the heart belongs to fire, while the spleen belongs to soil. Obesity takes spleen deficiency as the basic pathogenesis, and "deficiency nourishes the mother". Rhizoma coptidis tastes bitter, and the fire in the tastes is bitter, so it can nourish the fire and benefit the soil, and its heat-clearing ability can prevent warm medicines such as Ginseng from hurting the body and consuming qi. Ginger is piquancy, Jujube is sweet, "piquancy plus sweet can produce Yang", reconcile Ying and Wei, make the body function smoother, promote the absorption and utilization of drugs. The whole prescriptions can reinforce Qi, tonify Yang, invigorate spleen and eliminating phlegm.

III - II Drug preparation

Soak the medicine in water for 30min before decocting. The water needed for decocting should be soaked in the medicine for 3cm. When decocting, simmer it for 60min after boiling it with mild heat. Then pour the liquid out and place it at room temperature. Then add water to the decocting pan until the drug has been soaked for 1cm, simmer it again for 40min after boiling it. Combine the two filtrates, filter and concentrate with three layers of gauze.

IV Establishment of obese mouse model

90 SPF healthy C57BL / 6 male mice were randomly divided into 2 groups after 1 week of adaptive feeding: 15 normal mice were fed with normal diet, and 75 model mice were fed with high-fat diet. Body weight was measured weekly. Obese mice were screened after 10 weeks of high-fat diet, and those in the model group whose body weight was 20% higher than that of the normal group were considered as obese and successfully modeled.

V grouping of animals

In the model group, 62 mice were successfully established as obese mice, with a modeling rate of 82.7%. Fifty obese model mice were selected and randomly divided into five groups, namely model blank group, orlistat group, low dose TCM group, medium dose TCM group and high dose TCM group.

VI Drug intervention

According to the Pharmacological Experimental Methodology, the dosages were calculated by body weight. The dosages of the low, medium and high TCM groups were 11.76g/ (kg·d), 23.51 g/ (kg·d), and 47.02 g/ (kg·d) respectively. The dosages of the orlistat group were 0.091 g/ (kg·d). Reagent configuration after the corresponding concentration in glass bottles, 4 ℃. Every morning 8:00 drugs in 37 ℃ water bath after 20 minutes of experimental mice take corresponding drugs respectively, the normal group and model group were given corresponding blank volume of distilled water, for six weeks in a row, dose adjustments according to the weight of mice weekly. During the intervention, mice in the drug group were still fed a high-fat diet.

VII Detecting the serum biochemical indexes of mice

After 6 weeks of gavage and 2 hours after the last administration, 1% pentobarbital sodium was used to anesthetize the mice and blood was taken from their eyeballs. Let stand at room temperature, the use of ST16R type low temperature centrifuge at 4 ℃, centrifugal under the condition of 3500 RPM for 10 minutes, take supernatant, preserved - 20

℃. The levels of serum total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL-C) and low-density lipoprotein (LDL-C) were detected by Hitachi automatic biochemical analyzer in the laboratory department of affiliated hospital of Hebei University

VIII Measuring the fat volume of mice

The mice were anesthetized with 1% sodium pentobarbital, and then fixed on the mouse bed. Quantum FX microCT was used to scan the mice. After scanning, the obtained projection data were reconstructed and imported into the analysis software. The reference point was selected, and the density interval was adjusted. Only the portion of fat density was left on the screen and stained. It was divided into the area of interest and then superimposed on each layer to calculate the fat volume.

IX Statistical processing

All the data were statistically analyzed using SPSS 22.0 software, the measurement data were statistically analyzed using mean standard deviation ($\bar{x} \pm s$), and comparison between and two means was conducted using independent sample test, with $P < 0.05$ as the difference with statistical significance.

Results

I Weight

I - I Weight changes of mice during modelling

The weight changes of mice during the modeling period were shown in (Table 1). Before the modeling, i.e., 0 weeks, there was no significant difference in weight between the two groups *(P > 0.05)*. After 1 week of high-fat diet, the body weight of the model group was slightly higher than that of the normal group (*P < 0.05)*, but it did not reach the weight standard of the obese mouse model. At the end of 4 weeks, the weight of mice in the model group was 31.76% higher than that in the normal group. At the end of week 10, the body weight of the model group was 55.36% higher than that of the normal group (*P < 0.05)*.

Table 1: Weight changes of mice during modeling ($\bar{x} \pm s$)

Group	n	0 week（g）	1 week（g）	4 week（g）	10 week（g）
Normal group	10	16.73±1.04	19.88±1.14	23.68±1.09	27.24±1.20
Model group	10	16.35±1.27	22.67±1.39[a]	31.20±1.62[a]	42.32±2.58[a]
t		0.732	4.908	12.179	16.759
P		0.474	0.000	0.000	0.000

Note: [a]*P < 0.05* vs Normal group

I - II Weight changes of mice after drug intervention

As shown in (Table 2), before administration, there was no statistically significant difference in body weight between the model blank group, orlistat group and the low, medium and high dose Chinese medicine group (*P > 0.05)*, but the difference was significantly higher than that between the normal group and the model blank group (*P < 0.05)*. After 6 weeks of administration, the body weight of the orlistat group and the low, medium and high dose Chinese medicine group was lower than that of the model blank group (*P < 0.05)*. There was no difference among the low, medium and high dose groups (*P > 0.05)*, but the difference was higher than that in the orlistat group (*P < 0.05)*.

Table 2: Comparison of body weight of mice before and after drug intervention ($\bar{x} \pm s$)

Group	n	Before drug intervention（g）	After drug intervention（g）
Normal group	8	27.24±1.20	28.03±1.25
Model blank group	8	42.43±2.48[a]	47.02±2.61[b]
Orlistat group	8	42.18±2.26[a]	38.93±2.19[bc]
Low dose TCM group	8	41.23±3.54[a]	42.74±3.12[bcd]
Medium dose TCM group	8	42.04±2.32[a]	42.18±2.40[bcd]
High dose TCM group	8	41.76±2.63[a]	41.83±3.02[bcd]

Note: [a]*P < 0.05* vs Normal group (before administration), [b]*P < 0.05* vs Normal group (after administration), [c]*P < 0.05* vs Model blank group (after administration), [d]*P < 0.05* vs Orlistat group (after administration)

II Blood lipid level

As shown in (Table 3), compared with the normal group, the levels of TC, TG, LDL-C and HDL-C in serum of mice in the model blank group increased and decreased (*P < 0.05)*. Compared with the blank model group, there were significant differences in TG and HDL-C levels between the orlistat group and the blank model group (*P < 0.05)*. There were significant differences in TG levels in the low-dose group (*P < 0.05)*. TC and TG levels were significantly different in the medium dose group (*P < 0.05)*. TC, TG, LDL-C and HDL-C levels were significantly different in the high-dose group (*P < 0.05)*. Compared with the orlistat group, the HDL-C and LDL-C levels of the low-dose TCM group were significantly different (*P < 0.05)*. TC, TG, LDL-C and HDL-C levels were significantly different in the medium dose group and the high dose group (*P < 0.05)*. TC and TG levels in the medium dose group were significantly different from those in the low dose group (*P < 0.05)*. TC, TG, LDL-C and HDL-C levels were significantly different in the high-dose group (*P < 0.05)*. TC, HDL-C and LDL-C of the high-dose TCM group were significantly different from those of the medium-dose TCM group (*P < 0.05)*.

Table 3: Blood lipid level of mice after drug intervention ($\bar{x} \pm s$)

Group	n	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)
Normal group	8	2.18±0.16	0.71±0.12	1.50±0.19	0.37±0.08
Model blank group	8	3.92±0.53[a]	1.29±0.19[a]	1.21±0.22[a]	0.79±0.14[a]
Orlistat group	8	3.83±0.48[a]	1.04±0.14[ab]	1.43±0.16[b]	0.68±0.12[a]
Low dose TCM group	8	3.74±0.50[a]	1.08±0.14[ab]	1.19±0.23[ac]	0.82±0.12[ac]
Medium dose TCM group	8	3.06±0.38[abcd]	0.79±0.13[bcd]	1.20±0.27[ac]	0.81±0.11[ac]
High dose TCM group	8	2.63±0.27[abcde]	0.77±0.16[bcd]	1.47±0.21[bde]	0.57±0.10[abcde]

Note: [a]$P < 0.05$ vs Normal group, [b]$P < 0.05$ vs Model blank group, [c]$P < 0.05$ vs Orlistat group, [d]$P < 0.05$ vs Low dose TCM group, [e]$P < 0.05$ vs Medium dose TCM group

III Fat volume

As shown in (Table 4)

before the drug intervention, there was no statistically significant difference in fat volume between the model blank group, orlistat group and the low, medium and high dose groups ($P > 0.05$), but the fat volume of the five groups was higher than that of the normal group ($P < 0.05$).

After drug intervention, orlistat group and high, medium and low dose group mice fat volume were significantly lower than blank model group ($P < 0.05$), with orlistat group, the dose of Chinese traditional medicine group, high dose group were lower than low doses Chinese materia medica group ($P < 0.05$), the most significant effect of high dose of Chinese traditional medicine group reduce fat volume ($P < 0.05$).

Table 4: Comparison of fat volume before and after drug intervention ($\bar{x} \pm s$)

Group	n	Before drug intervention（cm^3）	After drug intervention（cm^3）
Normal group	8	6.68±1.57	7.37±0.93
Model blank group	8	14.37±0.91[a]	17.86±1.22[b]
Orlistat group	8	14.69±0.97[a]	11.76±1.05[bc]
Low dose TCM group	8	14.24±1.03[a]	12.83±0.84[bcd]
Medium dose TCM group	8	14.16±0.84[a]	11.75±1.12[bce]
High dose TCM group	8	14.36±0.95[a]	10.64±0.92[bcdef]

Note: [a]$P < 0.05$ vs Normal group (before administration), [b]$P < 0.05$ vs Normal group (after administration), [c]$P < 0.05$ vs Model blank group (after administration), [d]$P < 0.05$ vs Orlistat group (after administration); [e]$P < 0.05$ vs Low dose TCM medicine group (after administration), [f]$P < 0.05$ vs medium dose TCM medicine group (after administration)

Discussion

In this study, high-fat diet was adopted to induce obesity in mice, which was highly similar to most obese patients in simulating obesity attack path and was the most commonly used model for obesity research [3]. The results of this study showed that the weight of mice given orlistat and low, medium and high doses of Tranfat Decoction was lower than that of the model blank group. Both orlistat and transfat inhibited weight gain, with orlistat being the most effective. Since the mice in the model group were still fed with high-fat diet after the start of drug intervention, the weight of mice in the TCM group did not drop, but compared with the model control group, it can be seen that the weight of mice in the TCM group was well controlled, and the Tranfat Decoction was more mild than orlistat in weight control. It was found that the serum TC, TG, LDL-C of the obese mice fed with high-fat diet were significantly increased and HDL-C were decreased. High-dose Transfat Decoction can significantly reduce serum TC, TG, LDL-C levels and increase HDL-C levels in obese mice, while orlistat can only improve TG and HDL-C levels in mice, so Translipid Decoction is superior to orlistat in improving blood lipid levels. The decrease of TC, TG and LDL-C in serum indicates a high clearance of circulating lipids. Tranfat Decoction has certain advantages in preventing and treating the complications of obesity in blood metabolism [4].

In addition, a significant increase in adipose tissue volume was observed in mice fed a high-fat diet, but there was a decrease in adipose tissue volume in the orlistat group and the low, medium, and high dose Chinese medicine group, and the reduction effect was most significant in the high dose TCM medicine group. Adipose tissue is mainly composed of adipose cells, and the increase of fat volume is the reflection of excessive energy stored in adipose cells as triglycerides, indicating the increase of intracellular lipid accumulation [5]. Therefore, the mechanism of regulating the number and size of fat cells has become an important target of obesity research [6]. In this study, the effect of weight reduction in mice in the high dose TCM medicine group was not as obvious as that in the orlistat group, but it was better than that in the orlistat group in reducing fat volume. It can be seen that Tranfat Decoction can essentially treat obesity, which may be the reason why Chinese medicine treat obesity is not easy to recurrence.

To sum up, Transfat Decoction has an exact effect on the control of body weight in obese mice, and the high dose Transfat Decoction is even better than orlistat in improving blood lipid level and reducing fat volume.

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