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Research Article

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The effect of instant Pu-erh tea intragastric administration in mice with hydrogenated oil diet-induced obesity

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ABSTRACT

Obesity has become a fast growing epidemic in developing countries, as well as in some of the developing countries. Tea showed strong effect in reducing body fat and lowering blood sugar. Intragastric administration has the advantage of higher controllability and quantifiable compared with feeding. The diet-induced obese mice by intragastric administration need shorter time to show out weight loss effect. This article tested the effect of instant Pu-erh tea on weight loss in diet-induced obese (DIO) mice. The results demonstrated that instant Pu-erh tea was be able to reduce the mouse weight dose-dependently. Its effect is stronger than that of L-carnitine, a weight loss drug currently on the market. Instant Pu-erh tea also accelerated lipid metabolism and eased the high-fat diet-induced liver injury.

Key words: Obesity; Instant Pu-erh tea; Lipid-lowering; Weight losing effect

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INTRODUCTION

Obesity refers to excessive body fat tissue content. Currently, obesity is becoming a worldwide epidemic disease, and the incidence is increasing year by year. Looking for effective weight loss drugs or diets from natural products has become a research hotspot[1]. In recent years, a number of epidemiological studies have indicated that tea has certain anti-obesity effect [2].

The advantages of instant tea include high consistent quality, easy to quantitate, and convenient to use. Instant Pu-erh tea was made from Pu-erh tea, a traditional tea in Yunnan province, through extraction, filtration, concentration and drying processes. In this study, the obese mice induced by high fat diet were used to test the efficacy of instant Pu-erh tea on its weight loss and lipid-lowering abilities. The results showed that instant Pu-erh tea had multiple beneficial effects on these obese mice, including weight loss, blood lipid regulation, and prevention for obesity. This study provided the basis for the prevention and treatment of obesity with Pu-erh tea in human.

EXPERIMENTAL SECTION

2.1 Experimental animals and reagents

Kunming male mice (SPF), the initial body weight being 22 ± 2 g, were provided by the DaShuo Animal Science and Technology Co., Ltd. Jianyang. Certificate of conformity of the experimental animals: 0020699; license number: scxk (Chuan) 2008-24.

Instant Pu-erh tea was purchased from Econazole Biological Technology Co., Ltd. L-carnitine was used as positive control, which was purchased from, Shanghai Jing Pure Industries Limited.

2.2 High fat diet

Animal feedstuff was provided by the Suzhou Experimental Animal Feed Science and Technology Co., Ltd. High-fat diet was that of basal feed with hydrogenated oils as add-ons. The daily supply of hydrogenated oil of

mouse was calculated based on that a 70 kg adult intakes 30 g fat each day and 1.5 times of this quantity were applied.

2.3 Obesity model

90 SPF Kunming male mice were used for tests. The control group had 10 mice and were fed with normal diet. The obesity induction group (n = 80) were fed with high fat diet for a period of nine weeks. Mice weighting more than 20 % of the average body weight of the control group were accepted as obese mice and used for further experiments [3].

2.4 Experimental group design and dosimetry

The obese mice induced by high fat diet were randomly divided into six groups. The positive control group were fed with high fat feed containing L-carnitine, and the dose is in accordance with the research of Mun et al [4]. The negative control group were fed with high fat forage. Experimental mice were fed with high fat diet.

2.5 Feeding method

The mice were housed in a temperature of 20-22 °C, and humidity of 65-68 % of the experimental environment, maintaining the day light of 12 h. They were fed with basal feed for a week, making them adapt to the new environment and the way of taking food, to reduce the inconvenience and experimental error caused by the stress response.

During the trial, the control group was fed with basal diet, the other groups were fed with high fat diet.

2.6 Measure changes in mice weight

All of the mice were weighed once on the first day of the experiment, and then weighed twice a week. The change of average weight was calculated and recorded for each group.

Tab.1: The designing of experimental groups

Groups	Dose groups	Number of samples	Intake amount (g/ Kg.bw.d)	Feedstuffs
Control group(C)	-	10	Distilled water	BD
Negative control group(N)	-	10	Distilled water	HFD
Positive control group(P)	-	10	0.75	HFD
Instant Pu-erh green tea group(G)	G1	10	0.021	HFD
	G2	10	0.043	HFD
	G3	10	0.064	HFD
Instant Pu-erh tea group(R)	R1	10	0.021	HFD
	R2	10	0.043	HFD
	R3	10	0.064	HFD

(Note: BD, basic diet; HFD, High-fat diet; 1,2,3 represents low, medium and high dose groups.)

2.7 Determination of the mice blood and biochemical parameters of serum

At the 12th week of the experiment, after fasting for 12 hours, all mice were taken blood by the way of orbital venous plexus. The blood was kept at room temperature for 30 min, and centrifuged for 10 min at 3500 rpm. The supernatant were used to determine biochemical parameters. All measurements for serum biochemical parameters were carried out with commercial kits according to their instructions.

2.8 Mouse liver biopsies

Liver tissues were fixed overnight in 10 % neutral formalin. Tissue blocks were paraffin embedded. Slide sections were prepared and stained with H&E. Changes in liver tissue were observed under an optical microscope.

RESULTS AND DISCUSSION

At the beginning, there was no significant difference in the body weight of all groups of mice. After feeding with high fat diet containing hydrogenated oils for nine weeks, 10 mice were randomly selected as the negative control group (group N). The average weight of mice in group N was 21.76 % higher than that of group C, meaning the successful preparation of the obese model.

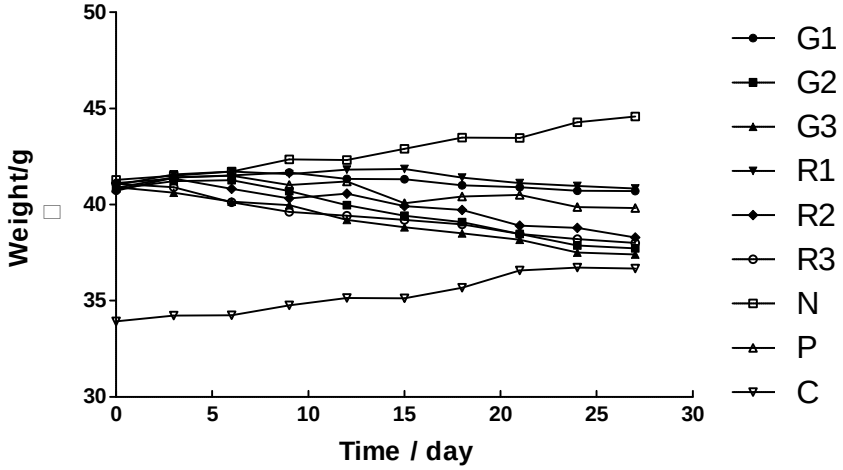

Fig.1: The average bady weight of each experimental group

(Note: C: Control group, N: Negative control group, P: Positive control group, G: Instant Pu-erh green tea group, R: Instant Pu-erh raw tea group. 1,2,3 represents low, medium and high dose groups.)

Tab.2: The effect of instant Pu-erh tea on body weight of experimental mice

Groups	Dose groups	Initial weight	Experimental final weight
Instant Pu-erh green tea group	G1	40.74±1.28	40.70±2.69
	G2	40.83±1.83	37.73±2.15**
	G3	40.63±2.04	37.42±2.97**
Instant Pu-erh ripe tea group	R1	41.03±1.79	40.83±2.43
	R2	41.48±2.75	38.28±3.09**
	R3	41.12±1.62	38.04±1.82**
Positive control group	P	40.86±2.05	39.82±3.48*
Negative control group	N	41.20±1.76	44.35±2.17
Control group	C	33.92±1.65	36.68±2.30

*(Note: Compared with the N group, * indicates P <0.05, ** indicates P <0.01)*

Giving test substance 27 days, it can be seen from Fig.1: Most of the mice given the test substance at the start of the weight there is a slight upward trend, which may be due to the small mouse is still in the growth stage. This may be due to mice weight will be increased due to the continued intake of high lipid diet. In the 6 days after administration of the test substance, the weight of mice began to decline. And with the extension of treatment time, weight loss effect is more obviously. L-carnitine was used as positive control, which has lower effect on body weight in mice. But the process of administration in mice is relatively large weight fluctuations.

As shown in Tab.2, after 27 days of the experiment, the body weight of group P was significantly ($p < 0.05$) lower than that of group N. Body weight of mice fed with feedstuff and intragastric administration instant Pu-erh tea was also lower than that of the group N. The weight loss effect of instant Pu-erh tea on obese mice was dose dependent. The best weight loss group was group G3 and R3, whose final body weight were even lower than the initial weight. The weight of the instant Pu-erh tea group also had the downtrend with the increasing dose. However, compared with the group N, the G1 and R1 group had no remarkable difference ($p > 0.05$). With the doses increasing, the G2,G3,R2 and R3 groups had a large difference ($p < 0.01$).

Various plant hydrogenated oils were heavily used in various occasions, such as peanut butter. There are also many items prepared with partially hydrogenated oil, margarine and vegetable oil. These hydrogenated oils contain trans fatty acids [5] and it has been shown that long-term intake of hydrogenated oils are harmful to human body [6].

Experimental mice were fed with feedstuff and intragastric administration instant Pu-erh tea for 27 days, and weighed twice a week. During this period, the weight of experimental mice changed obviously, and the average body weights of mice in group G and R were all lower than that of group N. Moreover, compared with the group P, the weight of mice intragastric administration instant Pu-erh tea was generally lower in a dose dependent manner. The result showed that instant Pu-erh tea did have a slimming effect, and the effect is better than that of L-carnitine, a body loss drug currently on the market [7].

At the 13th week of the experiment, after fasting for 12 hours, all mice were taken blood. The serum biochemical indexes were assayed and the liver tissues were processed and subjected to pathological study. Serum biochemical parameters have been analyzed, including total cholesterol (TC), triglyceride (TG), LDL-C and HDL-C levels, alanine transaminase (ALT), and aspartic transaminase (AST) activity [8]. The results showed in Fig.2, instant Pu-erh tea lowered the levels of TC, TG, and LDL-C in serum. At the same time, the treatment increased the level of HDL-C in serum and lowers the enzyme activity of ALT and AST.

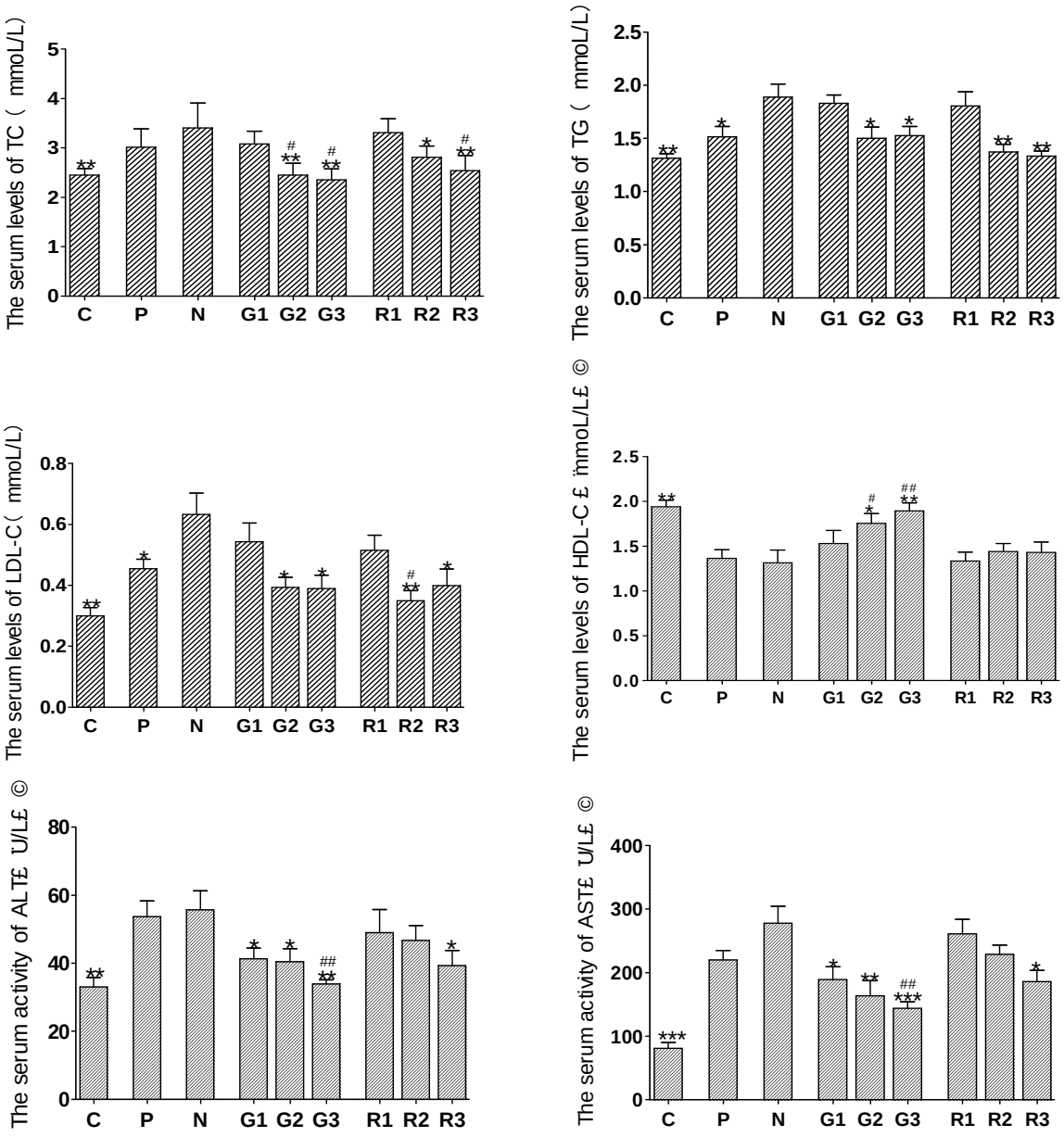

Fig.2: The serum activity in instant Pu-erh tea intragastric administration group

*(Note: C: Control group, P: Positive control group, N: Negative control group. 1,2,3 represents low, medium and high dose groups. Compared with the N group, * indicates P <0.05, ** indicates P <0.01, *** indicates P <0.001. Compared with the P group, #p<0.05,##p<0.01,###p<0.001.)*

With the treatment, the serum biochemical parameters could be close to the values of normal and healthy mice. As shown in Fig.3, these effects were consistent.

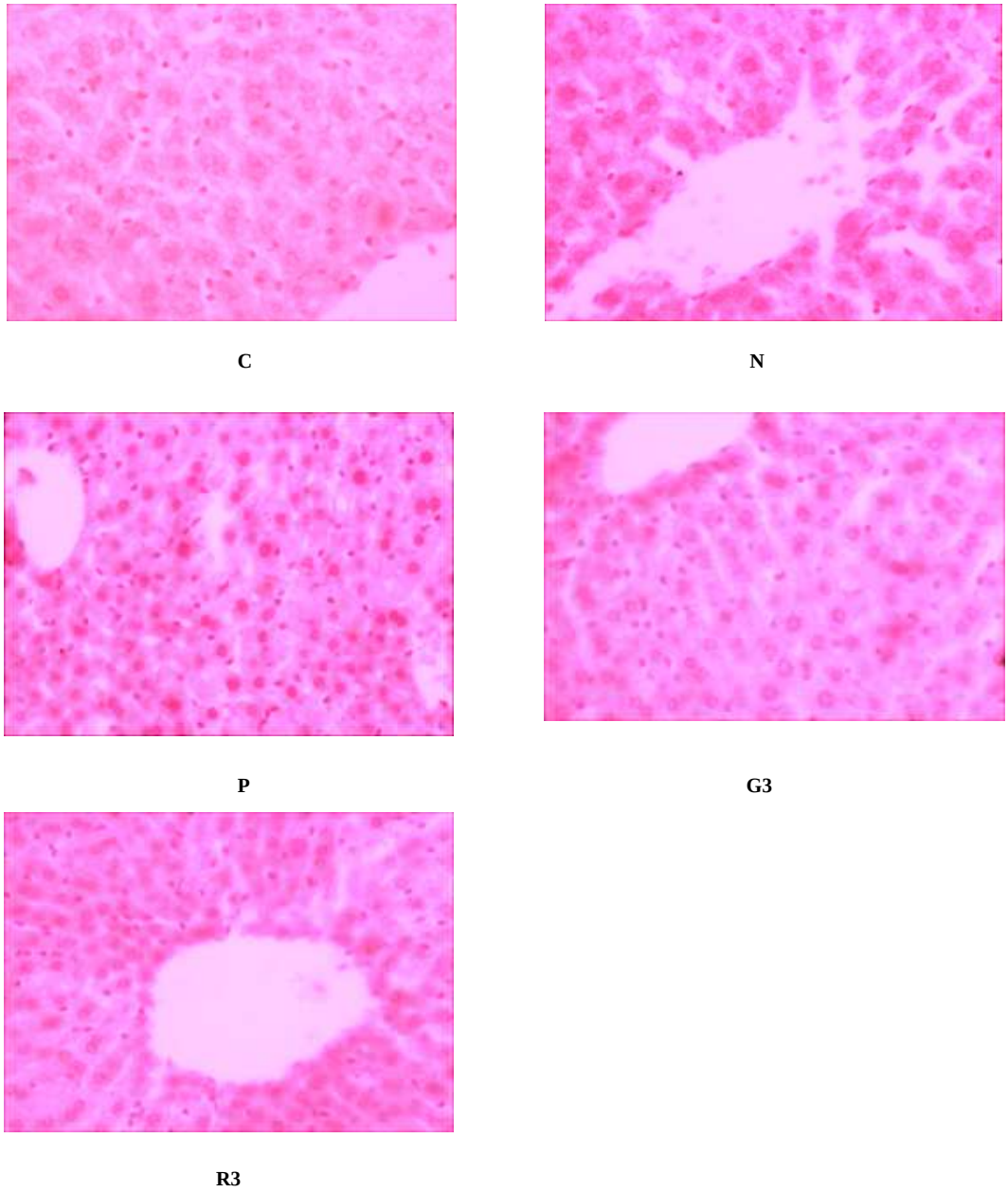

Fig.3: The liver histopathology of each experimental group
(Note: C: control group; N: negative control group; P: positive control group; R: instant Pu-erh tea group.)

Observed under a microscope, the livers of the tea treated mice had no obvious difference from the livers of control group C. There were no signs of fatty liver in these groups. In contrast, fatty liver diseases were the most serious in group N. Compared with group N, the mice liver tissue damage was minor in group P, but was still more serious than group C. The liver tissue injury in instant Pu-erh tea group was significantly lower. Their lobular morphology was essentially normal without any obvious pimelosis (Fig.3).

CONCLUSION

A long-term intake of high fat diet containing hydrogenated oils causes excessive weight gain in mice, resulting in the increasing of TG, TC, and LDL-C in serum. The results showed that instant Pu-erh tea decreased serum TC, TG, LDL-C and HDL-C levels and normalized ALT and AST activity. The more the instant Pu-erh tea in the diet of treated groups, the better the effect of weight loss on these mice, indicating that the tea was working in a dose-dependent manner [9]. Indeed, instant Pu-erh tea have a slimming effect, and the effect is better than that of current drug L-carnitine.

In this study, obese mice were induced by the diet containing trans fatty acids. This induction method mimicked the

occurrence of obese human by diet with excessive fatty acids. Previous studies on the weight losing effect of Pu-erh tea were generally carried out using tea extract [10]. However, there was a huge variation in the tea varieties, processing, storage and the leaching conditions on the tea extraction, etc. Thus, repeatability and guiding significance are limited in these studies. The current study used a solid instant tea of standardized content, which can guarantee the reliability of the experimental data and the standardization of the dietary intake. The effective dose range and optimum intake dosage of Pu-erh tea for weight losing and lipid-lowering of obese mice are studied and identified. This experiment not only confirmed that the high fat diet containing trans fatty acids was harmful to the human body, but also made a detailed research for the effective dosage of instant tea. Therefore, it provided a reference for further research on the obesity prevention and lipid-lowering efficacy of the Pu-erh tea, and laid the theoretical foundation for its application.

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