

History of Pu'er Tea and comparative study for the effect of its various extracts on lipid-lowering diet

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Abstract: Pu'er Tea is a kind of traditional historical famous tea which gains its name for native government jurisdiction in Pu'er (now Xishuangbanna in Yunnan, Pu'er city etc), and takes Pu'er (now Ning'er county of Pu'er city) city as its collecting and distributing center. It is famous all over the world because of its good benefits for reducing blood lipid, slimming weight, antibacterial, aid digestion, detoxification and other functions, it is even known as the health care beverage with "the fine quality goods for preserving people's health", "a health drink demanded everyday". Although there are a lot of current study literature about the effect of Pu'er Tea on lipid-lowering and reducing weight, but there is rarely contrast study about the effect of lipid-lowering diet with its various extracts. Therefore, this article uses the acetone, water, chloroform, ethyl acetate and n-butanol to continuously extract Pu'er Tea, then freeze and dry them into four major separate components which include the chloroform layer, ethyl acetate layer, butanol layer and the remaining water layer. Take advantage of different components for filling and feeding the ICR mice which are treated with the processing of obesity molding, then compare the extract of Pu'er Tea with the weight-loss drug L-carnitine which is popular all over the market, explore the slimming effect of each component in Pu'er Tea on the cells of ICR fat mice. The results show that the total water extract of Pu'er Tea, ethyl acetate extract, residual water extract all have obvious effect on reducing body weight and body fat of experimental mice, it also has significant lowering effect on blood lipid and liver lipid in mice, that could significantly inhibit the accumulation of lipid in fat cells and hypertrophy of fat cells, reveal that the Pu'er Tea has good function of lipid-lowering and reducing weight. At the same time, the comprehensive effect of lipid-lowering and reducing weight through Pu'er Tea is superior to commercial weight loss drug L-carnitine, and weight reducing effect of ethyl acetate extracts in Pu'er Tea and residual water layer is better than total water extract of Pu'er Tea. But n-butyl extract doesn't show a significant effect of lipid-lowering and reducing weight, inferring that efficacy component of Pu'er Tea which plays a major role of the effective component of lipid-lowering and reducing weight may exist in ethyl acetate extract and residual layer material.

Keywords: Pu'er Tea, history, effect of lipid-lowering, reducing weight.

INTRODUCTION

Pu'er Tea is a kind of traditional historical famous tea (Zhou, 2004), which gains its name for native government jurisdiction in Pu'er city (now Xishuangbanna in Yunnan). Pu'er Tea is a kind of special tea with unique shape and endoplasmic nature. Since Tang and Song dynasties, it had been flourishing for one thousand years, and still is an important kind of drink. As one of China's top ten famous tea, Pu'er Tea has now again become the special kind of tea which is esteemed by consumers in the tea market of southeast Asia, Hong Kong, Macao, Taiwan regions and Europe and the United States. Especially it has antibacterial, falling hematic fat, reducing weight, digestion, detoxification and other effects, it is highly appreciated in Japan, France, Germany, Italy, Hong Kong, Macao and other countries and regions.

With Rise in obesity and associated health consequences of obesity such as diabetes and dyslipidemia are on rise worldwide (Mackay *et al.*, 2010; WHO Media center, 2013). Ineffectiveness and side effects associated with prescription drugs for management of obesity has

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increased the public interest in the use of natural remedies such as Pu'er Tea. Lin's study etc found that, Pu'er Tea contains some non-existent compounds which does exist in green tea and black tea, these compounds produce strong inhibition to the generation of NO induced by lipopolysaccharide (LPS) in giant chew cell, they are considered to be the polymers of catechinic acid, but its chemical properties is not very clear, and the molecules which exert inhibition to NO is unknown (Lin *et al.*, 2003). Zhou Zhihong has obtained a new element of black tea pigment detached from Pu'er Tea (Puerins A and B) (Zhou *et al.*, 2005). L.S. Hwang etc obtained statins material detached from Pu'er Tea which could reduce the incidence of coronary heart disease and cerebrovascular disease through reducing blood fat and restraining inflammation (Hwang, 2003). Human hepatocellular carcinoma (HepG2) cells experiments have confirmed that the Pu'er Tea has the effect of reducing weight and lipid-lowering (Chiang *et al.*, 2005; Way *et al.*, 2009). Chen *et al* (2011) and Gong *et al* (2010)'s studies respectively reveal that theabrownin of Pu'er Tea can significantly reduce total cholesterol (TC), triglycerides (TG) and low density lipoprotein (LDL) levels in serum of rats with hyperlipemia, increase high-density

lipoprotein (HDL) levels, and reduce fat deposition in rat's liver, prevent the forming of fatty liver. Although there are a lot of current study literature about the effect of Pu'er Tea on lipid-lowering and reducing weight, but there is rarely contrast study for the effect of lipid-lowering diet with its various extracts. To this end, this paper introduces the contrast study for the effect of lipid-lowering and decreasing weight with Pu'er Tea through a brief introduction for its historical evolution path. Aim to further explore the key active ingredients of health benefits in Puer tea and provide reference direction and basis of health food research for the health food industry.

MATERIAL AND METHOD

Ripe Pu'er Tea was provided by research institute of Pu'er tea (Yunnan Pu'er). L-carnitine of luca element brand was commercially available. Kits for the assays of serum total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL - C), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), malondialdehyde (MDA) and protein were obtained from commercial sources.

Animals

Seventy-five male ICR mice (20±2g) were purchased from laboratory center of animal in Zhejiang university. Based feed of animals (M02-F) and fatty feed (M04-F) are purchased from Shanghai Slyke laboratory animal Co., LTD M02 -f basic group is divided into (%): 9.7% of it is moisture, 20.5% of it is crude protein, 4.6% of it is crude fat, 6.2% of it is crude ash, 4.3% of it is crude fiber, 50.6% of it is nitrogen free extract; 1.2% of it is calcium, 0.9% of it is phosphorus, 1.3% of it is lysine, 0.7% of it is methionine (cystine). Basic group of M04-F is divided into (%): M02-F54.6%, fat 16.9%, sugar 14%, casein 10%, maltodextrin 2.4%, sticky substance 2.1%. (Gong *et al.*, 2011)

The preparation for each separate component of Pu'er Tea

As shown in fig. 1, perform 60% of acetone extraction for 3 times (1 g tea is add to 4 ml of acetone solution) to Pu'er Tea at room temperature, then merger extraction, get the concentrated solution through removing acetone under the condition of reduced pressure with rotary evaporation, then adopt the chloroform, ethyl acetate, n-butanol and different polar solvent for extraction in succession. The Pu'er Tea is divided into four components: chloroform layer, ethyl acetate layer, n-butanol layer and residual water layer, though rotating, evaporation and making freeze drying in vacuum for 24h for each layer components, the obtained solids are kept at low temperature and set aside.

The method of experiment with animal

Before the trial, perform adaptive feeding to ICR mice in

clean barrier environment for 1 week, according to the weight, they are randomly divided into 2 groups, where the blank contrast group (CG) has 10 mice, which are fed with basic feed. The remaining 65 are belong to molding (MG) group, which are fed with high fat feed, and weigh the weight of each mice every week. After the success of the molding (40 d), randomly divide 60 mice into 6 groups according to the weight, each group has 10 mice, which are contrast group (OMG) of obesity model, l-carnitine group (LCG), group of total water extract from Pu'er Tea (PAG), ethyl acetate group (EAG), n-butyl alcohol (BAG) group and the remaining water layer group (SAG) respectively, and they are fed with high fat forage. The rats could get water and feeding freely during experiment, each experimental group are filled with corresponding dose (1.5mg/kg.bw) of corresponding extracts according to weight. Blank contrast and high fat model group are fed with distilled water. Experimental period are 8 weeks. During the experiment, weigh them every other day, keep records of intake and left intake amount. Fasting for 12 h at the end of the experiment, perform death to mice and quickly gather blood in mice. Perform anatomy to the mice, and observe the condition of fat and the changing status of liver, kidney, spleen and other major organs in mice, then make rapid separation of mice liver and body fat (testicles and perirenal fat pad), after making rinsing with physiological saline, blot the moisture and weigh them quickly. All samples are cryopreserved at - 20°C. Perform 2000 r/min centrifuge to blood at 4°C for 15 min, take the liquid supernatant for indicating biochemical index. Cut up the liver, then add the cold saline solution, perform homogenate at low temperature for 5 min with 1000 r/min, make 10% of the tissue homogenate, under 4°C, perform the centrifugal with 3000 r/min for 15 min, take the liquid supernatant for indicating biochemical index (Hwang *et al.*, 2003).

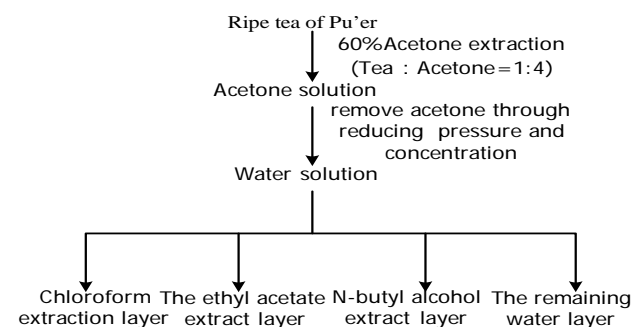


Fig. 1: Preparation for different extraction components of Pu'er Tea.

The detection for each component content of Pu'er Tea

Chromatograph of high performance liquid is SHIMADZU LC-2010A, test parameters are C18 column, column temperature is 35°C; Mobile phase A is glacial acetic acid: acetonitrile; heavy steam water (0.5: 3: 96.5); Mobile phase B is glacial acetic acid: acetonitrile; heavy steam water (0.5: 30: 69.5); phase B is given gradient

elution from 0 to 100% for 0-45 min; after 45 min ,phase B remains 100%, flow rate is 1 ml/min; the detection wavelength is 280 nm. External standard contrast has a gallic acid: (GA), GC, ECG, C, Caffeine, EC, EGCG, GCG, ECG, GC, Theaflavin, TF-3-G, TF-3'-G, TF-3, 3'-DG etc.

Detection of serum indicators in animal body

Serum total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL - C), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), lipid peroxidation products malondialdehyde (MDA), catalase (CAT) and coomassie brilliant blue protein all are determined by kit method. Food utilization ratio (the increase of body weight/food intake x 100%), coefficient of fat (perirenal fat + testis perirenal fat/animal weight x 100%) and atherosclerosis index (AI=(TC-HDL-C)/HDL-C) are all calculated with corresponding formula.

Data Processing and Analysis

The experimental data adopts Excel 2007 and Origin 8.0 software for data processing and analysis of variance, which is denoted with mean \pm standard deviation $\bar{x} \pm SD$. Using single factor analysis of variance, and compared it with the contrast group* denotes $P < 0.05$, which is the significant difference, ** denotes $P < 0.01$, which is extremely significant difference. (Mackay *et al.*, 2010)

RESULTS

Whole experiment process is performed in the animal barrier laboratories on the fourth floor in the animal experiment center of the Zhejiang University, laboratory environment control is good, temperature and humidity are within normal range. The experimental mice are found no obvious abnormality, their diet and activity is in good condition, face are black brown and in the form of long grain, urine is normal.

Content analysis of each component in Pu'er Tea

Though conventional test method for determination of Pu'er Tea, chloroform layer in the tea is mainly caffeine, its content is up to 82.3% of the dry matter, tea polyphenols content of ethyl acetate extract layer and n-butanol extraction layer is 54.7% and 33.5% respectively, main material of residual water is brown pigment of tea, polysaccharide, and part of protein, polyphenols etc, for the protein and polysaccharide after the precipitation of anhydrous ethanol, the brown pigment in residual water layer of tea is about 38.4% of the dry content. HPLC testing results show that the content of catechins and gallic acid in ethyl acetate extract layer is 16.4% and 2.8% respectively, and in n-butyl alcohol extraction layer, they are 3.3% and 0.6% respectively. Theaflavins is not found in acetic ether extraction and n-butanol extraction layer, it may be because that after fermentation in the

process of long-term storage, theaflavins changes into thearubigins, theabrownin and some high molecular compounds through oxidative polymerization.

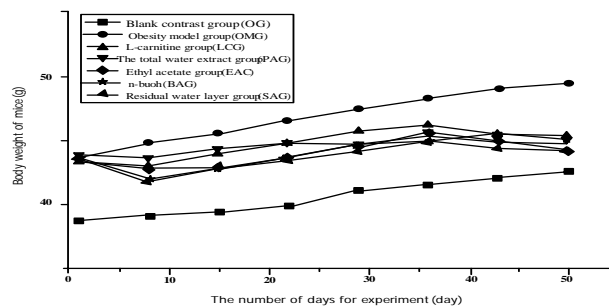


Fig. 2: The changes of mice body weight during the experiment.

Molding results in mice

Experimental results of molding (table1) shows that after the molding, the weight of mice in module group (MCG) increase significantly, as it is compared with the blank group (CG) there is extremely significant difference ($p < 0.01$). In terms of total food intake, total food intake of mice in the molding group have dropped significantly, but the utilization rate of food has greatly improved when compared with blank contrast group (the utilization rate is 8.48% and 12.81% respectively). That indicates high-fat feed has significant effect in promoting the nutritional obesity in mice, the result of experiment molding was a success.

The effect of different components of Pu'er Tea on body weight, food intake and food utilization of mice

As it can be seen from the fig. 2, body weight changing of mice in blank contrast group and obesity model group is more regular and near linear relationship, while the body weight of mice in the 5 experimental processing groups have obvious downward trend at the beginning of the experiment, this may be related to adapting ability of mice to new material, and it may be influenced by irrigation feed. Mice weight gradually rise and recover to the level at the beginning of the experiment at the middle of experiment, mice weight of each treatment group gradually decline at the end of the experiment, where the ethyl acetate and residual water group fall most obviously, although it does not reach significant difference when compared with obesity model group, but the weight value of mice has dropped to the level which is close to the level of the blank contrast group. Through comparing weight increment of mice in each processing group, we can find that the mice weight change of each experimental treatment group at the beginning and end of the experiment all have extremely significant differences with obesity model group and blank contrast group ($P < 0.01$) (table 2), where the weight increment in mice from ethyl acetate group and remaining water layer group is only 0.6 g and 0.4 g, they almost keep constant weight. The experiment results show that for the ICR mice in the

experiment, when feeding high fat forage, if feed a dose of medicine l-carnitine or Pu'er Tea extracts at the same time, it helps to curb the effect of high fat feed on weight gain in mice, which have certain effect on reducing weight.

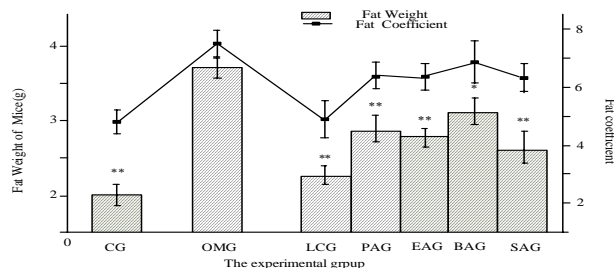


Fig. 3: The effect of different components in Pu'er Tea on fat weight and fat coefficient of mice.

On the other hand, the total food intake and food utilization ratio is also a key factor that result into the change of body weight in mice, in this experiment (table 2); apart from the mice in the part of the groups have phenomenon of scattering food; mice of the other group have no significant difference of food intake during the experiment. But there is some change among the utilization ratio of food in mice of different experimental treatment group, food utilization rate of obesity model group is the highest (3.61%), weight gain in mice is obvious; remove the blank contrast group (2.16%), diet pills L-carnitine group (1.18%), food utilization rate of total water extract in Pu'er Tea, ethyl acetate group, n-butanol and residual water group are relatively low, all of them are no more than 1%. It indicates that the effect of high-fat food on body weight in mice is not obvious, the reason may be that diet pills -L-carnitine or Pu'er Tea group can inhibit high fat feed conversion or absorption of mice respectively, which reflects the role of lipid-lowering diet, on the other hand it also suggests that the weight loss of mice in each group can not be reached by reducing the food intake.

The effect of different components in Pu'er Tea on the body fat of obese mice

The experimental results show that the weight of perirenal fat pad and testicular fat pad are significantly higher than that of blank contrast group, which proves that the high fat feedstuff can effectively promote the accumulation of body fat, its total fat weight has significant difference with blank contrast group ($P<0.01$) (fig. 3). After the experiment, only the fat pad weight of mice in n-butanol treatment group has obvious difference with obesity model ($P<0.05$), while the fat pad weight of the other L-carnitine group, ethyl acetate group, Pu'er Tea extract group and surplus water layer group shows extremely significant difference ($P<0.01$), especially the weight loss drug L-carnitine treatment group, the fat pad weight of mice tends to blank contrast group. Our experiment

results show that each component of Pu'er Tea can effectively inhibit fat accumulation in mice which are fed with high fat forage, its effects are less than the diet pills -L-carnitine, where the fat pad weight of mice in the residual water layer and ethyl acetate group drops more apparently, which shows Pu'er Tea has significant weight loss effect on inhibiting fat accumulation in the body of simple nutritional obesity mice.

On the other hand, fat coefficient is the ratio of total fat pad weight of mice with the weight of mice, it is also the important indicator of judging the degree of obesity in mice, from the data in fig. 3 we can see, on the aspect of reducing fat coefficient of mice, diet pills L-carnitine shows the best effect, its index value is basically close to the value of fat coefficient in blank contrast group, this may be related to its own properties (Kang *et al.*, 2011). At the same time, each component of Pu'er Tea also has a good effect on reducing fat coefficient of obese mice, which reaches a significant difference with the obesity model group ($P<0.05$), although there are significant difference when compared with the contrast group, but it has made a good effect on inhibiting further increase of fat index in mice and effectively improves the comprehensive index of mice.

The effect of different components in Pu'er Tea on serum, liver lipid of obese mice

Obesity is closely related to serum index and liver lipid level in the body, the rise of blood lipid may be the precursor of obesity. The results of this experiment (table 3) show that: compared with the blank contrast group, TC and TG of serum and liver in model group of obese mice all have significantly increased, which reach extremely significant difference ($P<0.01$); while the HDL-C index in serum decrease, and reflects the significant differences ($P<0.05$). It indicates that high fat diet can significantly promote the increase of lipid levels in mice. After the treatment of filling and feeding diet pill L-carnitine and different components of Pu'er Tea, the serum of mice, in addition to n-butanol of Pu'er Tea treatment group, TC and TG in the other treatment group decrease significantly, where the ethyl acetate treatment group and surplus water layer treatment group has the most obvious reducing effect on TC, although it could not recover TC index to normal level, but it has showed significant differences ($P<0.01$) when compared with obese group; but the total water extract of Pu'er Tea treatment has the most remarkable reducing effect on TG, ethyl acetate treatment group and surplus water layer treatment group is the second, their(including L-carnitine group) TG level is not only significantly lower than obesity model group, but also reach an extremely significant level when compared with the blank contrast group (table 3), which shows that weight loss drug L-carnitine and different ingredients of Pu'er Tea (except for butanol group) have significant effects on reducing the content of TG in serum

of obese mice. While on the aspect of improving the level of serum HDL-C in mice among every group, only the ethyl acetate group and surplus water layer treatment group show significant effect, the total water extract of Pu'er Tea treatment can improve the level of the HDL-C. Weight loss drug L-carnitine treatment group and n-butanol treatment group basically have no effect. In general, diet pills L-carnitine and each ingredient of Pu'er Tea (except for butanol group) have a certain effect on improving the serum indexes of obese mice, its improving effect on TG and HDL-C is superior to that of TC.

On the aspect of improving hepatic lipid levels of obese mice, it has certain distinction with the results observed in the serum, first, in terms of reducing TC and serum TC value in the mice of each experimental treatment group (with the exception of n-butyl alcohol group) are higher than that in the contrast group, TG is lower than the blank contrast group; while in the liver the situation is the opposite, TC value of liver in obese mice is lower than the contrast group, TG is higher than that of blank contrast group. Especially for the weight loss drug L-carnitine treatment group, focusing on the serum indexes, its effect of reducing TG in each experimental group is the worst, while the effect on reducing TG of liver is the best, which are related to that L-carnitine has the characteristics of carrying enzyme.

On the effect of the serum atherosclerosis index (AI) in obese mice, the AI value of fat model group is obviously 10 times higher than the blank contrast group, which indicates that feeding with the high fat diet can significantly increase the risk of atherosclerotic disease in mice. While after the process of experiment, the index value of atherosclerosis in mice from each experimental group has decreased significantly, especially the AI value of ethyl acetate treatment group and the remaining water layer group has reduced to be close to contrast group level, which indicates that some ingredients of Pu'er Tea has great potential on anti atherosclerosis.

The effect of different components in Pu'er Tea on serum and anti-oxidation index of liver in obese mice

The determination results on the effect of different components in Pu'er Tea on serum and antioxidant indexes of liver in obese mice are shown in fig. 4 that,

each component of Pu'er Tea can significantly improve the serum, anti oxidative enzymes SOD and GSH-PX activity of liver in mice, and reduce the content of lipid peroxidation malondialdehyde (MDA). Compared with the obese model group, processing the total water extract of Pu'er Tea, extract of ethyl acetate layer and residual water layer can significantly increase the serum and SOD, GSH-PX activity of mice, and significantly decrease the content of MDA at the same time. N-butanol extract treatment shows no relevant effect in all indexes; weight loss drug L-carnitine treatment reflects the significant

effect on rising hepatic GSH-PX activity of liver and reducing MDA content of mice ($P < 0.01$), it also has certain effect on improving the serum SOD activity of obese mice and decreasing the content of MDA, while it does not reflect obvious activity on other indexes.

The effect of different component in Pu'er Tea on fat cells in obesity mice

The effect of different component in Pu'er Tea on fat cells in obesity mice is as shown in fig. 5, the results show that the fat cells of mice in obesity model group become bigger, the size of fat cells are unequal and there is a certain degree of deformation, the number of fat cells within the same vision range through microscope decrease. Compared with the mice of obesity model group, after the treatment of total extract in Pu'er Tea, extract of ethyl acetate layer and surplus water layer, the diameter of fat cells in mice significantly reduce, the number of fat cells increase within the same view range, and the size of fat cells in mice from ethyl acetate treatment and residual water layer treatment group have been close to blank contrast group, it reveals that Pu'er Tea contains effective functional component which could reduce fat accumulation in fat cells and inhibit swelling of fat cells, that may exist in the extract of ethyl acetate or (and) remaining water layer .

DISCUSSION

In this paper, through using acetone, water, chloroform, ethyl acetate and n-butanol to perform continuous extraction of Pu'er Tea to get different components, and then combine with the commercial diet pills L-carnitine, to make comparative study for slimming effect of Pu'er Tea. The experimental results show that, the total water extract of Pu'er Tea, ethyl acetate extract, surplus water layer all have obvious reducing effect on weight and body fat of mice, it also has a significant lowering effect on blood lipid and liver lipid accumulation in mice, which can significantly inhibit the accumulation of lipid in fatty cells and hypertrophy of fat cell to reveal the good function of reducing weight through Pu'er Tea. (Zhou *et al.*, 2005) At the same time, comprehensive lipid-lowering effect of Pu'er Tea is better than the merchandise weight loss drug L-carnitine, weight loss effect of ethyl acetate extract in Pu'er Tea and surplus water layer extract is better than the total water extract of Pu'er Tea and n-butanol extract show no obvious slimming effect (Yang *et al.*, 2010), so we infer that the effective ingredients of Pu'er Tea which leads slimming effect may exist in the ethyl acetate extract and surplus water substances.

CONCLUSION

The experimental results show that ethyl acetate layer of Pu'er Tea and surplus water layer have stronger role of

weight reducing effect and improving antioxidant index than other separate set, therefore, the further separation of this part and studying the slimming effect will be the focus of our future research. The research contents of this

paper can provide reference direction and basis for development of new product for tea or health food industry.

Table 1: The effect of high fat diet to the molding of mice

Group	Body weight (g)			Food intake (g)	Food utilization ratio (%)
	Beginning weight (g)	Final weight (g)	weight increment (g)		
Blank contrast group (CG)	23.4±1.18	38.8±0.91**	15.4±1.1**	181.7±7.4*	8.48±0.15
Molding group (MG)	23.5±1.36	43.6±2.6	20.1±2.2	156.9±8.9	12.81±0.24

Note: "*" denotes that when it is compared with obese module: at P<0.05 level, it has significant difference "**" means significant difference at P<0.01 level

Table 2: The effect of different components of Pu'er Tea on body weight, food intake and food utilization of mice

Group	Body weight g			Food intake (g)	Food utilization ratio (%)
	Beginning weight (g)	Final weight (g)	Weight increment (g)		
Blank contrast group (CG)	38.8±0.91**	42.6±0.76**	3.8±0.18**	175.6±8.91	2.16±0.21
Obesity model group (OMG)	43.6±0.77	49.5±0.99	5.9±0.37	163.6±0.77	3.61±0.37
L-carnitine group (LCG)	43.4±0.87##	45.4±0.68	2.0±0.07***##	168.7±0.87	1.18±0.11
The total water extract group (PAG)	43.7±1.01##	45.0±1.21	1.3±0.05***##	153.5±1.01	0.85±0.06
Ethyl acetate group (EAG)	43.7±1.1##	44.3±0.88	0.6±0.1***##	163.7±1.1	0.37±0.04
N-butyl alcohol group (BAG)	43.5±0.93##	44.8±0.74	1.3±0.06***##	149.7±0.96	0.87±0.11
Residual water layer group (SAG)	43.8±1.0##	44.2±1.12	0.4±0.01***##	160.8±1.1	0.25±0.03

Table 3: The effect of different components in Pu'er Tea on serum index and liver lipid of obese mice $\bar{x} \pm SD$

Group	Serum index (mmol/L)				Liver lipid (mmol/L)	
	TC	TG	HDL-C	AI	TC	TG
Blank contrast group (CG)	1.90±0.21**	1.82±0.19**	1.55±0.15**	0.23±0.01	0.68±0.11**	0.75±0.07**
Model group (OMG)	5.66±0.9	2.36±0.26	1.28±0.14	3.42±0.55	0.99±0.22	2.24±0.39
L-carnitine group (LCG)	3.24±0.13***##	1.30±0.07***#	1.40±0.10	1.31±0.13***##	0.66±0.13**	0.94±0.21***#
The total water extract group (PAG)	3.21±0.11***##	1.02±0.07***##	1.55±0.23*	1.07±0.15***##	0.41±0.13***#	1.61±0.24***##
Ethyl acetate group (EAG)	2.98±0.23***##	1.13±0.16***##	2.07±0.35***##	0.44±0.06**	0.42±0.07***#	1.25±0.28***##
N-butyl alcohol group (BAG)	4.77±0.41##	2.12±0.10##	1.35±0.32	2.53±0.35***##	0.69±0.2**	1.81±0.52***##
The remaining water layer (SAG)	2.88±0.14***##	1.14±0.18***##	2.22±0.09***##	0.30±0.05***#	0.47±0.06***#	1.26±0.18***##

Note: "*" means when comparing obesity model of each group in the same column: it has significant difference at P<0.05 level, "**" means that at P<0.01 level, it has significant difference; "#" means that when comparing each group in the same column with blank contrast group, it has significant difference at P < 0.05 level, "##" shows significant difference at P<0.01 level.

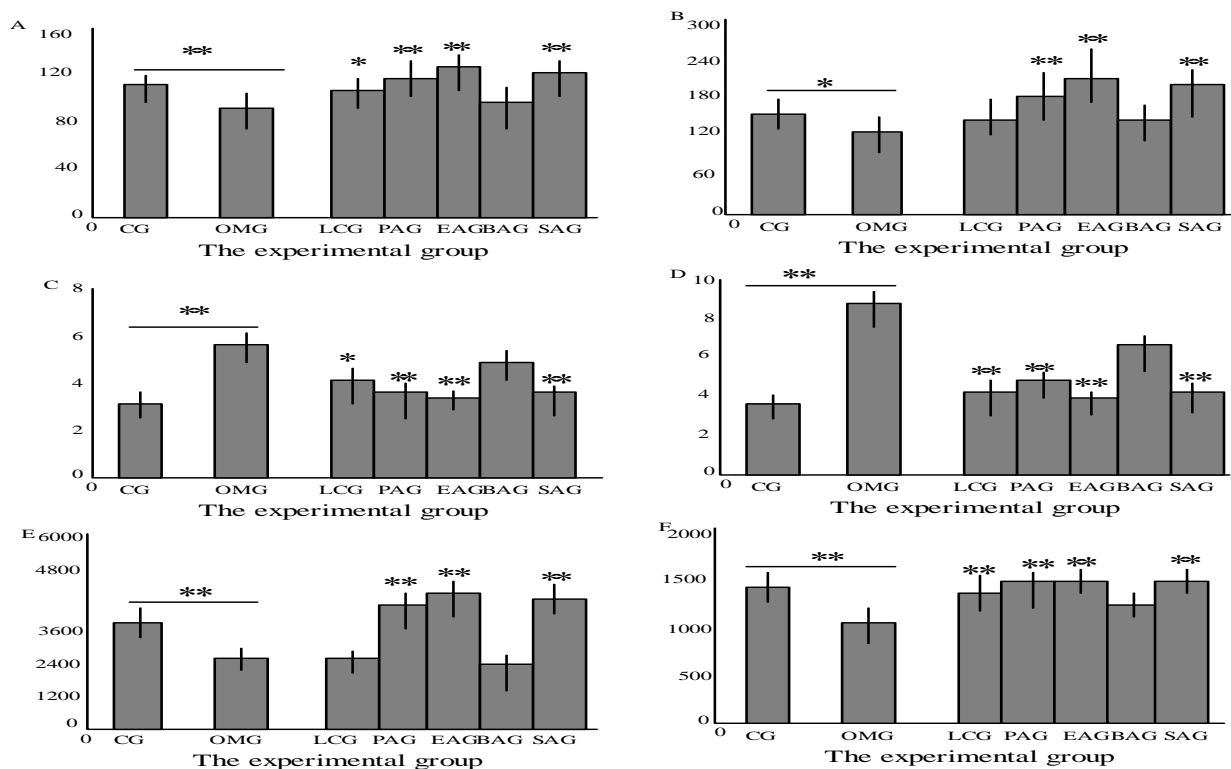


Fig 4: The effect of different components in Pu'er Tea on serum and liver SOD(A, B), MDA(C, D), GSH-PX(E, F) of mice

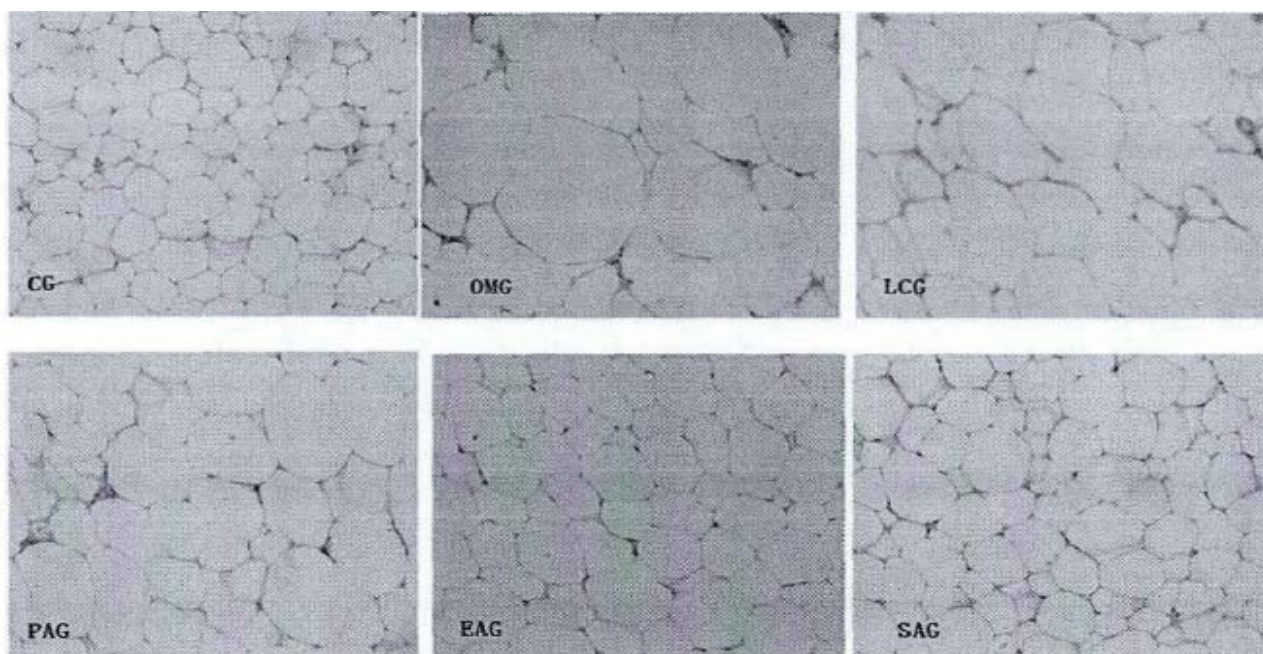


Fig. 5: The effect of different components in Pu'er Tea on fat cells in mice 400 x

Note: CG: blank contrast group; OMG: obesity model group; LCG: L-carnitine group; PAG: total water extract of Pu'er Tea group; EAG: ethyl acetate of Pu'er Tea group; BAG: n-butyl alcohol of Pu'er Tea group. SAG: remaining water layer of Pu'er Tea group

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