

THERAPEUTIC EFFECTS OF CHITIN FROM *PLEUROTUS ERYNGII* ON HIGH-FAT DIET INDUCED OBESITY IN RATS

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ABSTRACT

Background. Recent shifts in lifestyles and diets have caused the incidence of obesity to increase rapidly, resulting in a serious threat to modern human health. There is a growing interest the use of plant or fungi derived supplements as a safe and effective means to treat obesity. In recent times, edible-medicinal fungi have garnered attention as therapeutics owing to their biocompatibility and effectiveness. Attempts to determine the therapeutic effects of these fungi have become a prime focus in drug discovery programs. Therefore, this study aimed to determine the anti-obesity effects of *P. eryngii* chitin in rats with obesity induced by administration of a high fat diet.

Material and methods. To investigate the therapeutic effects of chitin from *Pleurotus eryngii* on high fat diet- induce obesity, we treated obese rats with different concentrations of chitin from *P. eryngii* for 4 weeks, using Lipitor as positive control. The living condition, food intake, body weight, perirenal adipose tissue, periepididymal adipose tissue, adipose tissue coefficient, serum lipid levels, including total cholesterol (TC), total glyceride (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C), were measured, and levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), themalonaldehyde (MDA), and superoxide dismutase (SOD) activity in liver were determined. The rats were also monitored for pathological changes in the liver and aorta.

Results. These studies indicated that administration of chitin from *P. eryngii* could significantly decrease obese rat food utilization rates and accumulation of adipose tissue in the body, thus preventing development of increased body weight. The treatment also significantly reduced serum lipid levels, including levels of TC, TG and LDL-C. Treatment with *P. eryngii*-derived chitin also enhanced ALT and AST enzymatic activity, enhanced SOD enzymatic activity, and reduced the MDA content of the liver, as well as significantly reducing the liver index and alleviating liver steatosis and aortic atherosclerosis resulting from obesity.

Conclusion. In conclusion, chitin from *P. eryngii* had therapeutic effects on hyperlipidemia, fatty liver, and aortic atherosclerosis resulting from obesity in rats.

Keywords: *Pleurotus eryngii*, chitin, obesity, therapy

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INTRODUCTION

Obesity is a type of systemic metabolic disease, not only causing changes in body shape, but also adding significant physical load and contributing to development and pathogenesis of many diseases (Huang et al., 2017). The deposition of a large amount of adipose tissue in organs, blood vessels, and other parts of the body, which can negatively affect cardio-cerebrovascular, hepatobiliary digestive, and respiratory system function, leading to hyperlipidemia, hypertension, diabetes, atherosclerosis, myocardial infarction, and other diseases (Gando et al., 2018; He et al., 2015; Li et al., 2016; Zhang et al., 2016). Lipoprotein levels are also disturbed or complicated with lipid metabolism disorders, accompanied by a reduction in HDL levels and an increase in total cholesterol (TC), triglyceride (TG) and LDL levels, which enter the liver and support fat synthesis, in turn causing development of fatty liver and abnormal liver function and elevated levels of associated liver transaminases (such as ALT, AST) (Han et al., 1999; Jahn et al., 2019; Merli et al., 2019). Fat accumulation in the liver will result in the overproduction of reactive oxygen species, activating the transcription of antioxidant genes, such as SOD and MDA, which help prevent hepatic steatosis through their antioxidant activities (Li et al., 2020a; Sena et al., 2018).

Chitin is a type of linear polysaccharide consisting of 2-acetylamino-2-deoxy-beta-D-glucose monomers linked by β -1,4 glycosidic bonds. Chitin in nature is a rare basic polysaccharide with a positive charge, typically found in the shells of insects and aquatic crustaceans, fungal cell walls, and algae; it is non-toxic, hydrophilic, bio-soluble, biodegradable, and has antibacterial characteristics (Jiang, 1996). Chitin has been reported to reduce caloric intake and accumulation of triglycerides in the liver, and lower serum lipid levels (Huang et al., 2016; Neyrinck et al., 2011; Zacour et al., 1992). Chitin exerts lipids-modifying effects mainly through regulation of cholesterol biosynthesis, metabolism, and efflux (Yang et al., 2019). Chitin has also been found to up-regulate expression of genes related to cholesterol excretion, including liver X receptor α , peroxisome proliferation-activated receptor- α , low-density lipoprotein receptor, and cholesterol 7 α -hydroxylase (Chiang, 2009), a rate-limiting enzyme of bile acid biosynthesis. Chitin plays

crucial roles in regulating circulating TC and LDL-C levels, and it can reduce expression of cholesterol synthesis genes, including 3-hydroxy-3-methylglutaryl-coenzyme A reductase, as well as regulate expression of genes related to cholesterol elimination and sterol-responsive element binding protein-2, a main regulator of control cholesterol biosynthesis gene expression (Liu et al., 2018).

Therefore, the goal of this study was to investigate the anti-obesity effects of chitin from *P. eryngii*. We established a high fat diet-induced obesity model to observe the effects of *P. eryngii* chitin on body fat content, hyperlipemia, liver function, obesity-induced atherosclerosis, and these findings illustrated that chitin from *P. eryngii* can reduce body weight gain, significantly reduce serum lipid levels, enhance hepatic function and antioxidant activity, significantly reduce the liver index, and alleviate liver steatosis and aortic atherosclerosis resulting from obesity. These findings suggest that chitin from *P. eryngii* could serve as a dietary alternative or supplement for the treatment of obesity.

MATERIALS AND METHODS

Materials

Chitin from *P. eryngii* were prepared by the Collaborative Innovation Center of the Mushroom Health Industry in Minnan Normal University. Lipitor (Pfizer Ireland Pharmaceuticals) is a type of clinical lipid-lowering drug used for the treatment of hypercholesterolemia and coronary heart disease with hypercholesterolemia.

72 male Sprague Dawley (SD) rats (SPF grade, license number: SCXK(Hu)2012-0002) weighing 100 ± 10 g were fed in different cages (4 rats per cage), under $22 \pm 2^\circ\text{C}$ and $55 \pm 10\%$ humidity with a 12 h light/dark cycle and food and water ad libitum for 5 to 7 d. High-fat diets included 1% cholesterol, 10% egg yolk powder, 10% lard, 0.2% cholate, and 78.8% basic diets and were sterilized using irradiation. The SD rats, high-fat diets and basic diets were purchased from Shanghai SLAC Laboratory Animal Co., Ltd.

TC, TG, HDL-C, LDL-C, ALT and AST quantification kits were purchased from Shenzhen Mindray Biomedical Electronics Co., Ltd. The SOD and MDA kits were purchased from Nianjing Jiancheng Bioengineering Institute.

Animal experiments

72 SD male rats were randomly divided into six groups (12 rats per group). The normal control group (NC) was fed with basic diet. The obesity model control group was fed with high-fat diet (HF). The obesity model treatment groups were fed with high-fat diet and included the Lipitor group (LP), the low-dose of *P. eryngii* chitin group (LPCG), the medium-dose of *P. eryngii* chitin group (MPCG), and the high-dose of *P. eryngii* chitin group (HPCG). Rats were fed with high-fat diet for 2 weeks, after which time the rat obesity model had established significantly different body weights when compared with the NC group (Li et al., 2020b). All experimental groups received intragastric administration of *P. eryngii* chitin or Lipitor according to a body weight ratio of 10 mL/Kg, LP (5 mg/(Kg·d)), LPCG (0.25 g/(Kg·d)), MPCG (0.5 g/(Kg·d)), and HPCG (1 g/(Kg·d)), while the NC and the HF groups were given the same dose of normal saline, with fasting for 2 hours prior to administration. Treatment was administered continuously for 4 weeks. The experiment was carried out according to the experimental setup (Fig. 1). We conducted regular daily administration of the relevant treatment and recorded body weight, food intake, and living conditions, replaced bedding and water every two days, and collected blood from the tail vein every two weeks to determine levels of serum biochemical indicators using an automatic biochemical analyzer.

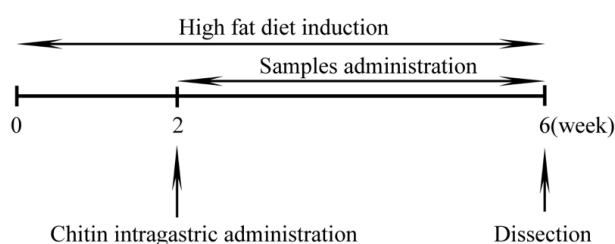


Fig. 1. Experimental setup

Method

Macroscopic and histological assessment, biochemical analyses. After administering the last treatment, we weighed the rats and collected blood from the tail vein after a 12 h fast. The blood was then centrifuged at 3000 rpm, 4°C for 5 min to obtain serum, and the

serum was used to analyze HDL-C, LDL-C, TG, TC, AST, and ALT content, which were determined using an automatic biochemical analyzer (BS-220, Mindry, Shenzhen, China). The rats were sacrificed by cervical dislocation and the heart, liver, spleen, thymus gland, periepididymal fat pad, and perirenal fat pad were harvested and weighed. The left hepatic lobule and aorta were fixed in 10% formalin, paraffin-embedded, and sliced into 4 μm sections for hematoxylin-eosin (H&E) staining in order to observe changes in histological structure. The right lobe of the liver was stored in liquid nitrogen until analyzed to determine SOD enzymatic activity and MDA content. We also calculated the food utilization rate, organ index and fat index according to the following formulas:

$$\text{Food utilization rate, \%} = \frac{\text{weight gain}}{\text{food intake}} \times 100$$

$$\text{Organ index, \%} = \frac{\text{wet organ weight, g}}{\text{body weight, g}} \times 100$$

$$\text{Fat index, \%} = \frac{(\text{perirenal fat weight} + \text{periepididymal fat weight}), \text{ g}}{\text{body weight, g}} \times 100$$

Statistical analysis

All data were showed as means ±SD, and all data were analyzed using SPSS 19.0 software (IBM, Ammon, New York, USA). Mean values were compared using one-way analysis of variance (ANOVA) followed by Tukey's-b test to compare means among the different treatment groups. A significant difference was accepted with $P < 0.05$.

RESULTS

Effects of chitin from *P. eryngii* on the growth status of rats

During the experiments, each group showed no obvious changes or gross abnormalities and displayed good diet and activity status. As shown in Figure 2, the NC group had bright and lustrous hair, robust activity, swift reactions, and uniform body carriage; while in rats in the HF group had rough, messy, and matted hair, carriage consistent with obesity, and obvious subcutaneous abdominal fat. Rats in the LP group presented with orderly and lustrous hair and uniform body

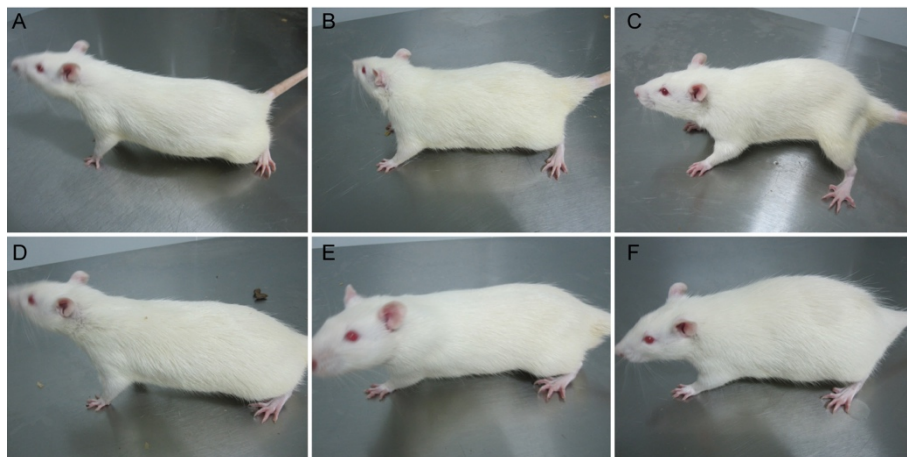


Fig. 2. Growth status for each experimental group of rats: A – NC, B – HF, C – LP, D – LPCG, E – MPCG, F – HPCG

carriage. The rats in the LPCG and MPCG groups appeared similar to those in the LP group, while rats in the HPCG group moved slowly, appeared to be obese, and had obvious subcutaneous abdominal fat.

Effects of chitin from *P. eryngii* on body weight and body fat content

As shown in Table 1, there were no significant changes in the HF group's body weight before and after modeling. After being fed with high-fat diets for 2 weeks,

each treatment group displayed significant differences in body weight compared with the NC group ($p < 0.05$). After treatment for 4 weeks, the body weight of the LP, LPCG, and MPCG groups significantly decreased when compared with the HF group (458.5 ± 19.6 g; $p < 0.05$). The weight loss was the most obvious in the LP group, with an average body weight of 403.0 ± 13.4 g, and the average weight was 419.5 ± 15.0 g and 426.8 ± 11.3 g for the LPCG and MPCG groups respectively, while the average body weight was not

Table 1. Effects of chitin from *P. eryngii* on body weight, weight gain and food intake of rats

Group	Number	Body weight, g				Food intake, g	Food utilization rate, %
		initial weight	weight after 2 weeks	final weight	weight gain		
NC	8	99.5 ±4.1	217.9 ±1.2	397.9 ±13.2	298.4 ±9.1	908.8 ±3.2	32.8 ±2.8
HF	8	104.5 ±5.8	232.3 ±2.1 ^a	458.5 ±19.6 ^b	354.0 ±13.8 ⁺	921.9 ±5.4	38.4 ±2.5
LP	7	96.1 ±2.4	234.5 ±2.4 ^a	403.0 ±13.4 ^d	306.9 ±11.0 ^{a,d}	910.2 ±4.6	33.7 ±2.4 ^c
LPCG	7	102.5 ±1.2	233.8 ±2.3 ^a	419.5 ±15.0 ^c	317.0 ±13.8 ^c	913.5 ±5.0	34.7 ±2.7 ^c
MPCG	8	96.1 ±2.2	232.4 ±1.8 ^a	426.8 ±11.3 ^c	330.7 ±9.1	939.5 ±6.2	35.2 ±1.5
HPCG	7	101.0 ±3.9	231.6 ±1.9 ^a	432.7 ±10.3	331.7 ±6.4	898.9 ±4.9	36.9 ±1.3

^aSignificant difference compared with NC ($P < 0.05$).

^bExtremely significant difference compared with NC ($P < 0.01$).

^cSignificant difference compared with HF ($P < 0.05$).

^dExtremely significant difference compared with HF ($P < 0.01$).

Table 2. Effects of chitin from *P. eryngii* on perirenal adipose tissue, periepididymal adipose tissue and adipose tissue index in rats

Group	Number	Perirenal adipose tissue g	Perirenal adipose tissue ratio of body adipose tissue %	Periepididymal adipose tissue g	Periepididymal adipose tissue ratio of body adipose tissue %	Total adipose tissue g	Adipose tissue index %
NC	8	5.60 ±0.8	1.39 ±0.11	5.48 ±0.77	1.37 ±0.41	11.08 ±1.57	2.77 ±0.79
HF	8	10.2 ±0.7 ^b	2.17 ±0.21 ^b	7.77 ±0.92 ^b	1.68 ±0.40 ^b	17.97 ±1.62 ^b	3.91 ±0.82 ^b
LP	7	8.28 ±0.59 ^{b,d}	1.43 ±0.15 ^{b,d}	4.09 ±0.49 ^{b,d}	1.01 ±0.12 ^{b,d}	12.37 ±1.08 ^d	3.06 ±0.80 ^c
LPCG	7	8.35 ±0.69 ^{b,d}	1.78 ±0.16 ^{b,d}	6.02 ±0.61 ^{b,c}	1.43 ±0.28 ^{b,c}	14.40 ±1.30 ^{a,c}	3.43 ±0.86 ^b
MPCG	8	8.91 ±0.87 ^{b,c}	1.82 ±0.23 ^{b,c}	6.93 ±0.97 ^b	1.62 ±0.35 ^b	15.84 ±1.84 ^{b,c}	3.71 ±0.62 ^b
HPCG	7	10.76 ±0.39 ^b	2.14 ±0.34 ^b	7.53 ±0.38 ^b	1.74 ±0.36 ^b	18.29 ±0.77 ^b	4.22 ±0.74 ^b

^aSignificant difference compared with NC ($P < 0.05$).

^bExtremely significant difference compared with NC ($P < 0.01$).

^cSignificant difference compared with HF ($P < 0.05$).

^dExtremely significant difference compared with HF ($P < 0.01$).

significantly decreased in the HPCG group and was similar to that of the HF group.

According to the food utilization rates in Table 1 and the average body fat measurements in Table 2, rats decreased their food utilization rate to reduce the accumulation of body fat in the LP, LPCG, and MPCG groups. The food utilization rate was 33.7 ±2.4%, 34.7 ±2.7%, and 35.2 ±1.5% in the LP, LPCG, and MPCG groups respectively, and the rates were all significantly decreased compared with the utilization rate of 38.4 ±2.5% in the HF group ($p < 0.05$). The perirenal adipose tissue, perirenal adipose tissue ratio of body adipose tissue, periepididymal adipose tissue, periepididymal adipose tissue ratio of body adipose tissue, total adipose tissue, and adipose tissue index were significantly decreased in the LPCG and MPCG groups, while there were no significant differences in body adipose tissue data between the HPCG and HF groups.

Effects of chitin from *P. eryngii* on serum lipid levels

As shown in Table 3, serum TC, TG and LDL-C levels were significantly higher, while HDL-C levels were significantly lower in the HF group due to lipid metabolic disorders resulting from consumption of a high-fat diet. After treating obese rats with chitin

Table 3. Effects of chitin from *P. eryngii* on serum lipids, mmol/L

Group	Number	Index			
		TC	TG	HDL-C	LDL-C
NC	8	1.38 ±0.13	0.54 ±0.20	1.07 ±0.09	0.24 ±0.04
HF	8	1.89 ±0.19 ^b	0.99 ±0.41 ^b	0.94 ±0.18	0.51 ±0.09
LP	7	1.62 ±0.26 ^{b,d}	0.54 ±0.15 ^b	0.91 ±0.08	0.34 ±0.05 ^{b,d}
LPCG	7	1.65 ±0.21 ^{b,d}	0.58 ±0.15 ^b	0.92 ±0.10	0.37 ±0.05 ^{b,d}
MPCG	8	1.69 ±0.18 ^{b,d}	0.63 ±0.30 ^c	0.93 ±0.07	0.41 ±0.07 ^{b,c}
HPCG	7	1.80 ±0.17 ^b	0.97 ±0.22 ^b	1.12 ±0.08	0.57 ±0.06 ^b

^aSignificant difference compared with NC ($P < 0.05$).

^bExtremely significant difference compared with NC ($P < 0.01$).

^cSignificant difference compared with HF ($P < 0.05$).

^dExtremely significant difference compared with HF ($P < 0.01$).

from *P. eryngii* for 4 weeks, the serum TC, TG, and LDL-C levels were significantly reduced in the LPCG

Table 4. Effects of chitin from *P. eryngii* on the organ indexes for each experimental group, %

Group	Number	Heart index	Spleen index	Liver index	Thymus gland index
NC	8	0.33 ±0.05	0.17 ±0.02	3.25 ±0.59	0.12 ±0.02
HF	8	0.31 ±0.02	0.17 ±0.02	4.56 ±0.61 ^b	0.13 ±0.02
LP	7	0.31 ±0.02	0.19 ±0.03	3.27 ±0.12 ^d	0.15 ±0.01
LPCG	7	0.33 ±0.04	0.19 ±0.03	3.67 ±0.07 ^d	0.12 ±0.03
MPCG	8	0.33 ±0.02	0.16 ±0.02	3.76 ±0.25 ^c	0.12 ±0.02
HPCG	7	0.32 ±0.01	0.18 ±0.02	4.01 ±0.54 ^b	0.13 ±0.04

^aSignificant difference compared with NC ($P < 0.05$).

^bExtremely significant difference compared with NC ($P < 0.01$).

^cSignificant difference compared with HF ($P < 0.05$).

^dExtremely significant difference compared with HF ($P < 0.01$).

and MPCG groups, while no significant changes in HDL-C levels were observed. Moreover, there was no significant difference in serum lipid levels between the HPCG and HF groups.

Effects of chitin from *P. eryngii* on the organ index

As shown in Table 4, there was no obvious difference in heart index, spleen index, and thymus gland index among the experimental groups. We did observe significant differences in liver index among the experimental groups, which suggested that high-fat diets induced hepatomegaly in rats. Compared to the HF group (4.56 ±0.61%), the liver index significantly reduced in the LP group (3.27 ±0.12%; $P < 0.01$), and was similar to that of the NC group, which indicates that Lipitor treatment can reduce accumulation of fat in the liver. The liver index was also significantly decreased in the LPCG and MPCG groups, which revealed that chitin from *P. eryngii* can similarly reduce accumulation of lipids in the liver.

Characterization of liver appearance

As shown in Figure 3, the liver appeared bright red, uniform in color with sharp edges, and lacking adipose tissue on its surface in the NC group. In the HF group, the livers were significantly larger than those of the NC group and showed yellowish-brown, blunt edges and irregular adipose tissues deposition under capsules with greasy sections. In contrast, livers from the

LP group appeared to be more red, uniform in color, and with partial blunt edges. The liver was slightly red, less uniform in color, and slightly greasy in the LPCG and MPCG groups. However, in the HPCG group, livers appeared yellowish-brown, dim, and greasy with partial blunt edges.

Histological changes of liver

As shown in Figure 4, in the NC group the hepatic lobular structure was intact, the hepatic sinus was clearly visible, the hepatic cords were in order, hepatocytes displayed normal morphology, were arranged linearly, and had no lipid droplets present. In the HF group, the liver exhibited severe steatosis, enlarged hepatocytes with many large lipid droplets present in the endochylema, a narrowed hepatic sinus resulting from compression, and disordered hepatic cords. In the LP group, the liver had intact hepatic lobules, a clearly visible hepatic sinus, regular hepatic cords, hepatocytes with normal morphology and some individual hepatocellular enlargement, and a narrowed hepatic sinus resulting from compression. Livers from the LPCG group exhibited intact hepatic lobules, a clearly visible hepatic sinus, regular hepatic cords, slightly enlarged hepatocytes, and a narrowed hepatic sinus resulting from compression. In the MPCG group, the hepatic lobules were partially destroyed, hepatocytes were slightly enlarged, and few lipid droplets were present. In the HPCG group, the liver displayed severe steatosis, hepatocytes were enlarged with numerous

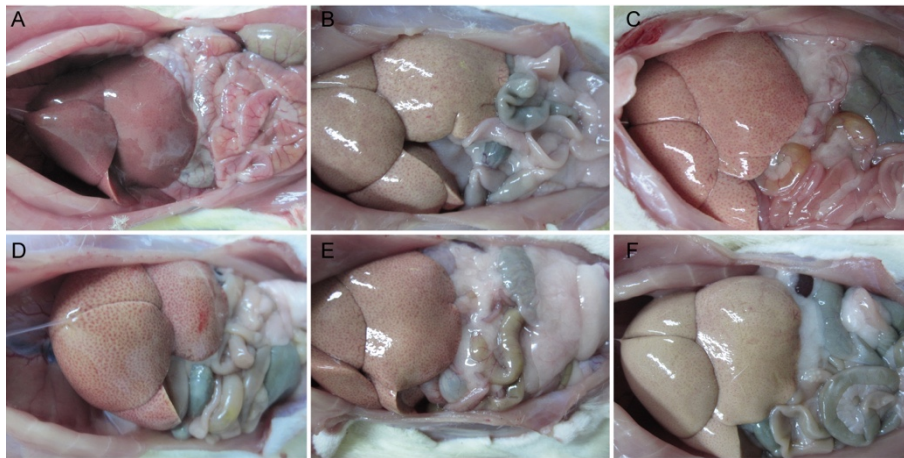


Fig. 3. Changes in liver appearance among rat experimental groups: A – NC, B – HF, C – LP, D – LPCG, E – MPCG, F – HPCG

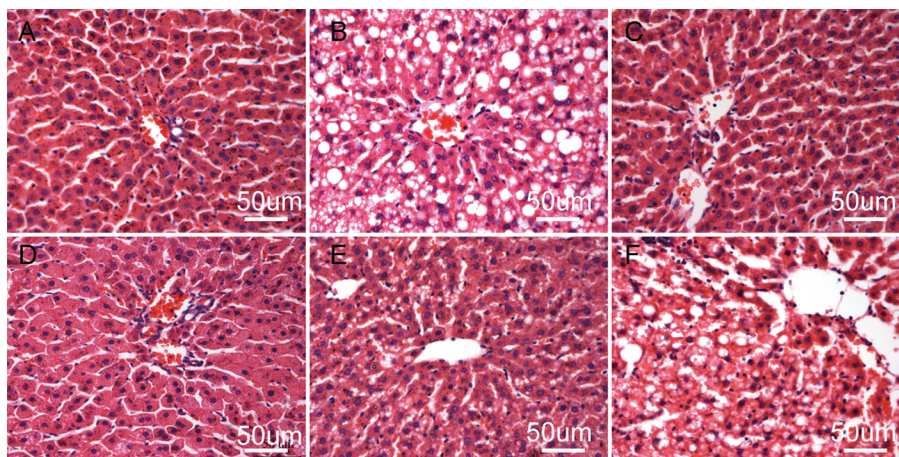


Fig. 4. Changes in liver histology among rat experimental groups (H&E, 400×): A – NC, B – HF, C – LP, D – LPCG, E – MPCG, F – HPCG

large lipid droplets present, the hepatic sinus was narrowed due to compression, and the hepatic cords were disordered.

Effects of chitin from *P. eryngii* on biochemical indexes of rat liver function

Abnormal lipid metabolic activity resulting from consumption of high-fat diets contributed to the production of large amounts of free fatty acids, which entered the liver to be synthesized into fat, ultimately resulting in the development of fatty liver and abnormal liver

function, in which serum ALT and AST levels were abnormally enhanced. Furthermore, liver MDA content was abnormally increased and SOD enzymatic activity was reduced. As shown in Table 5, compared with the HF group, serum ALT and AST levels were significantly decreased, SOD activity was increased, and MDA content was reduced in the LPCG and MPCG groups ($P < 0.01$). This resulted in improved liver function to promote proper lipid metabolism, thus reducing the accumulation of lipids in the liver and decreasing the incidence of fatty liver.

Table 5. Effects of chitin from *P. eryngii* on biochemical indexes of rat liver function

Group	Number	Index			
		ALT, U/L	AST, U/L	SOD, U/mgprot	MDA, nmol/mgprot
NC	8	33.81 ±0.70	79.41 ±0.72	15.08 ±1.37	4.35 ±1.49
HF	8	45.10 ±0.93 ^b	126.1 ±4.39 ^b	8.19 ±3.25 ^b	10.75 ±3.77 ^b
LP	7	34.75 ±0.68 ^d	81.17 ±0.39 ^d	18.23 ±2.60 ^d	4.91 ±1.33 ^d
LPCG	7	35.77 ±0.40 ^d	85.05 ±0.64 ^{a,d}	17.28 ±1.35 ^d	5.43 ±1.93 ^{a,d}
MPCG	8	37.52 ±0.74 ^{a,d}	95.58 ±1.40 ^{b,d}	14.51 ±2.01 ^d	9.14 ±1.85 ^b
HPCG	7	42.88 ±0.95 ^b	118.8 ±3.21 ^b	11.87 ±1.29 ^{b,d}	16.87 ±1.56 ^{b,c}

^aSignificant difference compared with NC ($P < 0.05$).

^bExtremely significant difference compared with NC ($P < 0.01$).

^cSignificant difference compared with HF ($P < 0.05$).

^dExtremely significant difference compared with HF ($P < 0.01$).

Effects of chitin from *P. eryngii* on aorta

Pathological observation of rat aorta. As shown in Figure 5, in the NC group, the arterial intima, media, and outer membrane were intact with clear layers, in which the intima consisted of a monolayer of flat endothelial cells and their matrix, the media membrane consisted of long fusiform smooth muscle cells arranged neatly surrounded by collagen and elastic fibrous tissue, and the outer membrane was loose connective tissue. In the HF group, the arterial wall was obviously thickened and the elastic fibers had widened, and its growth was perpendicular to the surface of the intima or spreading out in many directions, the internal elastic lamina was tortuous, the elastic fiber layers were basically clear with obviously increased layers, the intima displayed uneven hyperplasia, the endothelial cells were swollen, and infiltration of T lymphocytes was also found under the intima. In the LP group, the arterial intima, media, and outer membrane were intact with clear layers, but the intima displayed uneven hyperplasia and swollen endothelial cells. In the LPCG group, arterial intima, media and outer membrane were intact with clear layers without obvious lesions. In the MPCG group, we found that the arterial intima, media, and outer membrane were intact with clear layers and the media membrane was slightly thickened due to smooth muscle proliferation, but no infiltration of T lymphocytes was found under the intima. We observed significant arterial wall thickening,

broadening of the elastic fibers, uneven hyperplasia of the intima, swollen endothelial cells, and infiltration of T lymphocytes under the intima in the HPCG group.

DISCUSSION

Obesity refers to excessive body adipose tissue deposition due to changes in physiological and biochemical functions, causing weight gain and a series of pathological changes of body. In this study, an obesity model was established by feeding rats high-fat diets. Significant adipose tissues accumulated in the bodies of the obesity model rats, especially around the kidneys and epididymis. Their body weight rapidly increased and contributed to development of obesity and slow movement. Total adipose tissue and adipose tissue index were 17.97 ± 1.62 g and $3.91 \pm 0.82\%$ respectively in the HF group, which were significantly higher than those of the NC group (11.08 ± 1.57 g and $2.77 \pm 0.79\%$). After treating obese rats with *P. eryngii* chitin, adipose tissue accumulation was significantly reduced when compared with the HF group around the kidneys and epididymis in the LPCG and MPCG groups, and their body weight was also significantly decreased, and were 419.5 ± 15.0 g and 426.8 ± 11.3 g respectively, when compared with the HF group (458.5 ± 19.6 g) These findings were in accordance with those reported by Choi (2002), which found that chitin could effectively reduce obesity-induced weight gain.

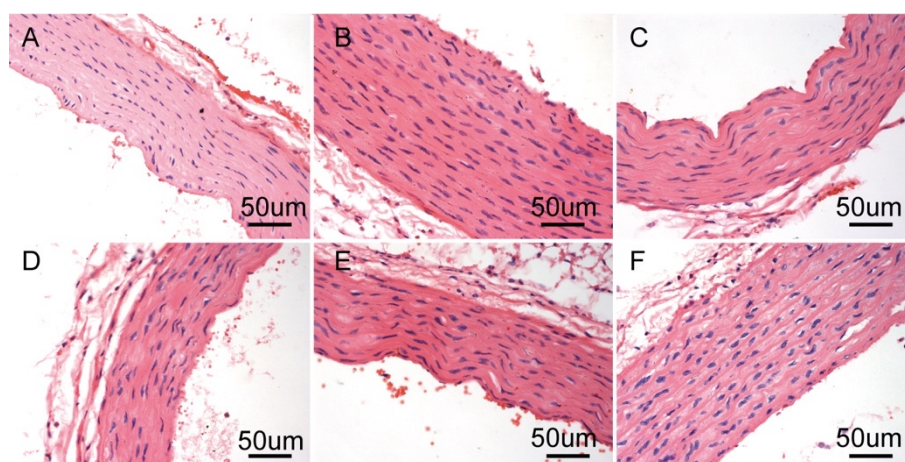


Fig. 5. Pathological changes in the aorta among rat experimental groups (H&E, 400×): A – NC, B – HF, C – LP, D – LPCG, E – MPCG; F – HPCG

Long-term intake of high-fat, high-cholesterol diets lead to a substantial increase in exogenous fat, which gets absorbed from the small intestine into the bloodstream and results in increased chylomicron production (Qiao et al., 2014). When the amounts of triglycerides synthesized in the liver are more than the capacity of hepatocyte transport, hyperlipidemia will occur, resulting in abnormally high serum TG and TC levels. Compared with the NC group, the TC and TG levels in the HF group were significantly higher, 1.89 ± 0.19 mmol/L and 0.99 ± 0.41 mmol/L respectively, and the LDL-C level was also increased. These findings are consistent with those reported by Zhong et al. (2000). In this study, the chitin from *P. eryngii* was used to treat the obesity model rats for 4 weeks, after which hyperlipemia was significantly relieved, and the TC, TG, and LDL-C levels were reduced to 1.65 ± 0.21 mmol/L, 0.58 ± 0.15 mmol/L, and 0.37 ± 0.05 mmol/L, respectively in the LPCG group. Similarly, the serum levels of TC, TG, and LDL-C were reduced to 1.69 ± 0.18 mmol/L, 0.63 ± 0.30 mmol/L, and 0.41 ± 0.07 mmol/L in the MPCG group. Treatment with chitin can improve lipase activity (Kang et al., 2012), reduce adipocytokine levels (Hsieh et al., 2012), down-regulate the expression of fat transcription factors (through modulations of the phosphorylated adenosine monophosphate-activated protein kinase (AMPK) and aquaporin-7 signal pathways) (Kong et al., 2011) to reduce TC, TG and LDL-C levels in serum to promote reduced accumulation of fat

in the body. We next performed studies to investigate the possible mechanism behind the changes observed with chitin treatment.

The liver is a crucial organ for fat metabolism. When a high-fat diet is consumed, levels of exogenous fat will increase in the body and the level of TG synthesized in hepatocytes will also increase, while serum TG levels will be reduced as TG flux into the liver increases. Excessive intake of cholesterol and free fatty acids will cause cytotoxicity and damage to hepatocyte function, resulting in decreased TG transport ability in hepatocytes. The above results will appear as abnormal liver function (Merli et al., 2019), in which ALT and AST levels are abnormally enhanced in the serum. Compared with the NC group, the ALT and AST levels in the HF group were significantly higher, 45.10 ± 0.93 U/L and 126.1 ± 4.39 U/L, respectively. Following 4 weeks of treatment with *P. eryngii* chitin, the ALT and AST levels in the LPCG and MPCG groups were significantly decreased. The fat deposited in the liver will cause the liver's change. Compared with the NC group, livers in the HF group were significantly larger, and showed yellowish-brown, blunt edges and irregular adipose tissue deposition under capsules with greasy sections, and the HF group also displayed severe steatosis, enlarged hepatocytes with many large lipid droplets in the endochylema, a narrowed hepatic sinus resulting from compression, and disordered hepatic cords. All of the above symptoms

were consistent with studies performed by Xing and Huang et al. (Hsieh et al., 2012; Huang et al., 2016; Xing et al., 2004). Following 4 weeks of treatment with *P. eryngii* chitin, serum ALT and AST levels were significantly reduced, hepatic SOD enzymatic activity was significantly improved, and the liver index and MDA content in the liver were decreased. Therefore, treatment with chitin can relieve liver steatosis and had a therapeutic effect on fatty liver.

Among the many factors contributing to atherosclerosis, hyperlipidemia is a major risk factor. Excessive lipids are deposited in the intima, which then locally form slightly raised lesions, which are then enclosed and fixed by hyperplasia of endometrial fibrous connective tissue to ultimately form atheromatous plaques. Increasing or enlarged plaques contribute to arterial wall sclerosis and narrowing or occlusion of the lumen, which can cause local tissue ischemia, coronary heart disease, cerebral thrombosis, and renal failure (Kang et al., 2012). In our study, rats in the HF group exhibited varying degrees of hyperlipidemia and aortic atherosclerosis. In the HF group, the arterial wall was obviously thickened and the elastic fibers were widened, and its growth was perpendicular to the surface of the intima or spreading in many directions, the internal elastic lamina was tortuous, the elastic fiber layers were basically clear with obviously increased layers, the intima displayed uneven hyperplasia, the endothelial cells were swollen, and infiltration of T lymphocytes was also found under the intima. After treating obese rats with chitin from *P. eryngii* for 4 weeks, the atherosclerosis of the aorta was significantly relieved. This finding was consistent with those reported in a study by Bays et al. (2013) where they found that administration of chitin could reduce the risk of aortic atherosclerosis.

CONCLUSIONS

In summary, obesity was induced in rats via administration of high-fat diets, and the anti-obesity activities of *P. eryngii* chitin and Lipitor were compared by analyzing biochemical variables including food utilization rate, accumulation of adipose tissue in the body, organ indexes, histological changes, blood lipid levels, and serum transaminase levels. We found that administration of *P. eryngii* chitin could reduce obesity,

decrease the liver index, decrease serum levels of TC, TG, ALT, and AST, improve SOD activity in the liver, decrease MDA content in the liver, and relieve liver steatosis. These findings indicate that *P. eryngii* chitin can mitigate numerous effects of obesity, including decreasing lipid levels, exerting a therapeutic effect on fatty liver, and reducing the risk of aortic atherosclerosis of aorta.

REFERENCES

- Bays, H. E., Evans, J. L., Maki, K. C., Evans, M., Maquet, V., Cooper, R., Anderson, J. W. (2013). Chitin-glucan fiber effects on oxidized low-density lipoprotein: a randomized controlled trial. *Eur. J. Clin. Nutr.*, 67, 2–7.
- Chiang, J. Y. (2009). Bile acids: Regulation of synthesis. *J. Lipid Res.*, 50, 1955–1966.
- Choi, H. G., Kim, J. K., Kwak, D. H., Cho, J. R., Kim, J. Y., Kim, B. J., ..., Choo, Y. K. (2002). Effects of high molecular weight water-soluble chitosan on in vitro fertilization and ovulation in mice fed a high-fat diet. *Arch. Pharm. Res.*, 25(2), 178–183.
- Gando, Y., Sawada, S. S., Kawakami, R., Momma, H., Shimada, K., Fukunaka, Y., ..., Blair, S. N. (2018). Combined association of cardiorespiratory fitness and family history of hypertension on the incidence of hypertension: a long-term cohort study of Japanese males. *Hypertens Res.*, 41, 1063–1069.
- Han, L. K., Kimuraand, Y., Okuda, H. (1999). Reduction in fat storage during chitin-chitosan treatment in mice fed a high-fat diet. *Int. J. Obes.*, 23(2), 174–179.
- He, Y., Zeng, Q., Zhao, X. L. (2015). Association of body mass index and waist circumference with risk of hypertension and diabetes in Chinese adults. *Med. J. Chin. PLA*, 40(10), 803–808.
- Hsieh, Y. L., Yao, H. T., Cheng, R. S., Chiang, M. T. (2012). Chitosan reduces plasma adipocytokines and lipid accumulation in liver and adipose tissues and ameliorates insulin resistance in diabetic rats. *J. Med. Food*, 15(5), 453–460.
- Huang, H. M., Wang, R. W., Li, S., Miao, A. Q., Xu, H., Tang, Q. (2017). Relationship between body mass index and percent body fat in the diagnosis of obesity: based on diagnostic tests. *Chin. J. Sports Med.*, 36(3), 218–225.
- Huang, J. F., Lin, J. F., Ou, Y. X., Liu, J. N., Cao, N., Zhuang, S. Y., ..., Pan, Y. T. (2016). Preventive effect of chitin from *Pleurotus eryngii* on fatty liver in rats. *Food Sci.*, 37(05), 219–222.

- Jahn, D., Dorbath, D., Schilling, A. K., Gildein, L., Meier, C., Vuille-Dit-Bille, R. N., ..., Geier, A. (2019). Intestinal vitamin D receptor modulates lipid metabolism, adipose tissue inflammation and liver steatosis in obese mice. *Biochim. Biophys. Acta Mol. Basis Dis.*, 1865(6), 1567–1578.
- Jiang, T. (1996). Chitin. Beijing: China Environmental Science Press.
- Kang, N. H., Lee, W. K., Yi, B. R. (2012). Modulation of lipid metabolism by mixtures of protamine and chitoooligosaccharide through pancreatic lipase inhibitory activity in a rat model. *Lab. Anim. Res.*, 28, 31–38.
- Kong, C. S., Kim, J. A., Bak, S. S., Byun, H. G., Kim, S. K. (2011). Anti-obesity effect of carboxymethyl chitin by AMPK and aquaporin-7 pathways in 3T3-L1 adipocytes. *J. Nutr. Biochem.*, 22(3), 276–281.
- Li, H. N., Zhao, L. L., Zhou, D. Y., Chen, D. Q. (2020a). Ganoderma lucidum polysaccharides ameliorates hepatic steatosis and oxidative stress in db/db mice via targeting nuclear factor E2 (erythroid-derived 2)-related factor-2/heme oxygenase-1 (HO-1) pathway. *Med. Sci. Monit.*, 26, e921905.
- Li, J., Wu, H., Liu, Y., Yang, L. (2020b). High fat diet induced obesity model using four strains of mice: Kunming, C57BL/6, BALB/c and ICR. *Exp. Anim.* <https://doi.org/10.1538/expanim.19-0148>
- Li, Z., Qin, W., Li, L., Wu, Q., Wang, Y. (2016). The combined effect of hyperuricaemia and overweight/obesity on risk of hypertension in adults. *WIMJ Open.*, 3(1), 10.
- Liu, S. W., Wu, Z. Y., Guo, S., Meng, X. J., Chang, X. D. (2018). Polyphenol-rich extract from wild *Lonicera caerulea* berry reduces cholesterol accumulation by mediating the expression of hepatic miR-33 and miR-122, HMGCR, and CYP7A1 in rats. *J. Funct. Foods*, 40, 648–658.
- Merli, M., Lattanzi, B., Aprile, F. (2019). Sarcopenic obesity in fatty liver. *Curr. Opin. Clin. Nutr. Metab. Care*, 22(3), 185–190. <https://doi.org/10.1097/MCO.0000000000000558>
- Neyrinck, A. M., Possemiers, S., Verstraete, W., De, B. F., Cani, P. D., Nathalie, M. D. (2011). Dietary modulation of clostridial cluster XIVa gut bacteria (*Roseburia* spp.) by chitin–glucan fiber improves host metabolic alterations induced by high-fat diet in mice. *J. Nutr. Biochem.*, 23(1), 51–59.
- Qiao, Y., Sun, J., Xia, F. S., Tang, X., Shi, Y. H., Le, G. W. (2014). Effects of resveratrol on gut microbiota and fat storage in a mouse model with high-fat-induced obesity. *Food Funct.*, 5(6), 1241–1249.
- Sena, C. M., Cipriano, M. A., Botelho, M. F., Seica, R. M. (2018). Lipoic acid prevents high-fat diet-induced hepatic steatosis in Goto kakizaki rats by reducing oxidative stress through Nrf2 activation. *Int. J. Mol. Sci.*, 19(9), :2706.
- Xing, X. B., Yan, X., Zhang, X. L. (2004). Effects of sibutramine upon steatohepatitis in obesity and hyperlipidemia rats. *Chin. J. Rehab. Med.*, 19(5), 369–391.
- Yang, D., Hu, C. J., Deng, X. Y., Bai, Y., Cao, H., Guo, J., Su, Z. Q. (2019). Therapeutic effect of chitoooligosaccharide tablets on lipids in high-fat diets induced hyperlipidemic rats. *Molecules*, 24(3), 514.
- Zacour, A. C., Silva, M. E., Cecon, P. R., Bambirra, E. A., Viera, E. C. (1992). Effect of dietary chitin on cholesterol absorption and metabolism in rats. *J. Nutr. Sci. Vitam.*, 38(6), 609–613.
- Zhang, L., Yan, Y. J., Wen, R., Liang, Y. X., Ren, L. J. (2016). Association between overweight, obesity, central obesity and cardiovascular diseases. *Mod. Pre Med.*, 43(21), 3887–3891.
- Zhong, L., Fan, J. G., Wan, G. L., Wu, W. Q., Shi, Q. (2000). The establishment of nonalcoholic steatohepatitis (NASH) animal model. *Lab. Anim. Sci. Adm.* 17(2), 16–20.