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Alleviation of high-fat-diet induced obesity and cholesterol accumulation in mice by extracts from male zooid of *Antheraea pernyi*

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ABSTRACT: In this paper, extracts from male zooid of *Antheraea pernyi* (EMZAP) were investigated for their anti-obesity activity in C57BL/6 male mice. Mice were randomly divided into 4 groups and fed on basal control group diet (Control Group), high-fat diet (Model Group), high-fat diet containing high dose of EMZAP (High Group), and high-fat diet containing low dose of EMZAP (Low Group), respectively. The levels of serum lipid, antioxidant capacity, body weight, and adipose weight were measured. The levels of expression of genes involved in cholesterol metabolism were also determined by Real-Time PCR. The results showed that EMZAP could reduce body weight and epididymal adipose weight of mice on fed high-fat diet, and also decrease serum lipid levels in mice with induced obesity. Treatment with EMZAP also produced significant amelioration of lipid accumulation and improved the antioxidant capacity in the liver. EMZAP reduced the expression of HMG-CoA reductase (HMG-CoA-R) and increased the expression of cholesterol 7-alpha hydroxylase (CYP7A1) and LDL receptor (LDLR). But it has no obvious effect on the expression of LXRα. According to the above results, EMZAP had good anti-obesity attributes and improvement in conditions arising out of cholesterol accumulation.

KEYWORDS: *Antheraea pernyi*, high-fat-diet, anti-obesity, cholesterol accumulation

INTRODUCTION

With the development of modern society, high-fat diets are becoming a popular dietary style. However, such incorrect components of diets may cause many health problems including obesity. Obesity is a major risk factor for dyslipidemia, type 2 diabetes, atherosclerosis, hypertension, cardiovascular diseases, and certain cancers in the developed world [1, 2]. Generally, these diseases are caused by a high level of cholesterol and triglycerides in the body [3]. From the view of metabolism, obesity and overweight status are caused by energy imbalance, which means the body absorbs more energy than it really needs [4]. The World Health Organization (WHO) points out that about 300 million people are obese (body mass index [BMI] > 30.0 kg/m^2) and 1 billion people are overweight (BMI of 25–30 kg/m^2) in the world [5]. The complications arising out of obesity are a worldwide concern because of the high costs of treatment and productivity losses.

About 75% of the body's cholesterol is synthesized and metabolized in the liver and small intestine. The reverse cholesterol transport (RCT) pathway mainly occurs in the liver [6]. HMG-CoA-R is the main rate-limiting enzyme for cholesterol synthesis. HMG-CoA-R is a precursor participating in the cholesterol synthesis in the cytosol and the endoplasmic reticulum [7]. LDLR is mainly responsible for transferring LDL-C from blood to the liver. The process includes a multistep enzymatic reactions [8]. CYP7A1 is the rate-limiting enzyme in the bile acid biosynthetic pathway that converts cholesterol into bile acids in the liver [9]. Liver X receptor (LXR), a member of the nuclear receptor super family of ligand-activated transcription factors, plays a central role in the regulation of cholesterol absorption, efflux, transportation, excretion, and many other processes related to lipid metabolism as well as inflammation [10].

Pure preparations from male zooid of *Antheraea pernyi* have long been used as a traditional Chinese medicine to treat many illnesses and promote longevity. The functions of these preparations are well documented in Compendium of Materia Medica. The functions are as follows: invigorating essential Qi, strengthening the vagina, no tiring during sexual intercourse, and arresting essence. It is believed that preparations from male zooid of

Antheraea pernyi can aid the liver and invigorate the kidney, consolidate essence, check bleeding, and promote muscle growth [11]. The male zooid of *Antheraea pernyi* contains many active substances, such as the brain hormone, prothymosin, hormone of *Antheraea pernyi*, and diuretics, all of which can adjust metabolism and restore immune functions [12]. Previous studies demonstrated that a concentrated liquor of male zooid of *Antheraea pernyi* could improve immune function, promote recognition, and cause lethal effects toward tumor cells. The liquor possessed a unique property for adjunctive therapy against tumors [13]. Moreover, the administration of the concentrated liquor of male zooid of *Antheraea pernyi* was suggested to help improve the immune function of the radiated tumor-bearing rats and reverse radiation-induced immune inhibition by promoting the proliferation of T helper cells and inducing their transdifferentiation from Th2 to Th1 [14]. There has been a lack of functional evaluation research on male zooid of *Antheraea pernyi* in China and worldwide. Therefore, we have developed new extracts of male zooid of *Antheraea pernyi* (EMZAP) and studied their curing properties and health care functions. EMZAP produced from newly emerged and unmated tussah moths were clear and transparent, free of turbidity, and excellent in appearance and flavor [15]. Our previous studies showed that these EMZAP could reduce the glutamate-pyruvate transaminase level in serum and reduce the degree of denaturation and necrosis of liver cells in mice when livers were injured by carbon tetrachloride. Thus, it was proved to have obvious protective effects on liver injury [16].

In this paper, we investigated the improvement of anti-obesity effects and cholesterol accumulation of EMZAP in high-fat-diet-induced mice. The body weight and liver weight were observed; and serum lipid analysis, biochemical analysis, and histopathological analysis were performed. In order to explore the effect of EMZAP on cholesterol accumulation, we studied the expression of genes related to cholesterol metabolism in the liver. All of these results are expected to provide a theoretical basis for the high-value utilization of tussah moths.

MATERIALS AND METHODS

Materials and animals

The fresh male zooid of *Antheraea pernyi*, which had been just hatched, was obtained from our own silkworm production base. The serum lipid and antioxidant capacity analysis kits were purchased from the Nanjing Jiancheng Bioengineering Institute. Trizol Total RNA Isolation Kit was purchased from Sangon Biotech (Shanghai) Co., Ltd.

A total of 40 male C57BL/6J mice, 6 weeks old with an average weight of 19 g each were purchased from Jinfeng Experimental Animal Co., Ltd. (SCXK 2014 0006) and housed under a 12:12 h light-dark cycle at 22 ± 2 °C and 40–60% humidity. All mice were maintained under pathogen-free conditions, provided *ad libitum* access to food and water, and treated humanely throughout the experimental period in strict accordance with the Shandong Institute of Sericulture guidelines for the care and use of laboratory animals.

Preparation of EMZAP

The fresh male zooid devoid of the wings were soaked in 95% alcohol for 7 days. After crushing and squeezing, the mixture was stored in the laboratory at constant temperature and humidity for 5–6 days. And then the mixture was centrifuged at 4200 rpm for 12 min. The supernatant was the raw dilute extracts of moths. The samples were concentrated under low temperature (liquid temperature 38 °C–42 °C) and negative pressure (−0.10 MPa to −0.08 MPa) to obtain porridge-like extracts of moths. After concentration, the samples were immediately centrifuged at 4000 rpm for 8 min and the precipitates and fat were removed. Then, 95% ethanol was added to the supernatant and kept it at −18 °C for approximately 6 h. After that, the mixture was centrifuged at −5 °C, 5000 rpm for 15 min [15]. The supernatant was the final EMZAP, to be used in the experimental animals after most of the alcohol evaporated. The final concentration was 0.8 g/ml. The specific process was shown in Fig. S1.

Animal experimental design

After acclimatizing for 5 days, the mice were randomly divided into 4 groups (of 10 each) and fed on basal control group diet (Control Group), high-fat diet (Model Group), high-fat diet containing high dose of EMZAP (High Group), and high-fat diet containing low dose of EMZAP (Low Group), respectively. The Control Group was fed with normal animal food. The other groups were fed with high-fat animal food. Mice were weighed and administered these diets daily via stomach gavage for 8 weeks until sacrifice. The Control Group and the Model Group were given normal saline by gavage once a day. The High and the Low Groups were given EMZAP by gavage 0.1 ml/10 g body weight and 0.05 ml/10 g body weight, respectively. After 8

weeks, these mice were fasted 12 h before sampling blood and tissues for analyses. The blood samples were obtained by removing the eyeballs. Blood was allowed to sit for 1 h to obtain the serum and then centrifuged at 4000 rpm for 5 min. The liver and epididymal adipose tissues were isolated after the mice were sacrificed. A part of the tissues was frozen in liquid nitrogen and stored at −80 °C until subsequent analyses and the other part was soaked in 10% formaldehyde solution for histopathological analysis.

Serum lipid analysis

Total-cholesterol (TC) and triglyceride (TG), HDL-cholesterol (HDL-C), and LDL-cholesterol (LDL-C) in the serum were measured with commercial kits.

Antioxidant capacity in liver

Each sample used for the measurement of liver enzyme activities was prepared by homogenizing the liver in a 0.9% NaCl buffer. The supernatant of the homogenate was obtained by centrifuging at 5000 rpm for 10 min at 4 °C. The protein content of liver tissue homogenate was measured with total protein quantitative kit (BCA colorimetry). The levels of T-AOC (Total antioxidant capacity), SOD (Superoxide Dismutase), MDA (Malondialdehyde), and GSH-Px (Glutathione peroxidase) in the supernatant were measured with commercial kits. The units of T-AOC, SOD and GSH-Px were U/mg protein. The unit of MDA was nmol/mg protein.

Histopathological analysis

For the histopathological examination, the liver and epididymal adipose tissues from each group were collected and fixed in 10% buffered formaldehyde solution for 48 h and embedded into paraffin. Then, the fixed tissues were sectioned and stained with hematoxylin and eosin (H&E) for histopathological evaluation.

Real-time PCR analysis

Approximately 100 mg of liver tissues were collected. Total RNA was extracted by using a Trizol Total RNA Isolation Kit, according to the manufacturer's instructions. Reverse transcription was carried out using RevertAid Premium Reverse Transcriptase (Thermo Scientific™ EP0733). The reaction was performed at 25 °C for 10 min, 50 °C for 30 min, and then 85 °C for 5 min. Synthesized cDNA was used for PCR with specific primers at optimized cycles. Primers were designed with the assistance of Sangon Biotech (Shanghai) Co., Ltd. as follows: GAPDH (Forward: 5′GGTTGTCTCCTGCGACTTCA 3′, Reverse: 5′TGGTCCAGGGTTTCTTACTCC 3′); HMG-CoA-R (Forward: 5′AGCGGAGCAGGCTAAGGTT 3′, Reverse: 5′TTGAGGTCACGACGGGAGA 3′); LDLR (Forward: 5′TCAGTCCCAGGCAGCGTAT 3′, Reverse: 5′CTTGATCTTGGCGGGTGTT 3′); LXRα (Forward: 5′CCTCAATGCCTGATGTTTCTC 3′, Reverse: 5′CTGACTCCAACCCTATCCCTAA 3′); and CYP7A1 (Forward: 5′GGGGATTGCTGTGGTAGTGA 3′, Reverse: 5′TGACAGGGAGTTTGTGATGAAG 3′).

Real-time PCR was conducted in an ABI Stepone Plus Real time PCR system (Applied Biosystems, USA) and SG Fast qPCR Master Mix (Sangon Biotech (Shanghai) Co., Ltd.). The reaction was performed at 95 °C for 3 min, followed by 40 cycles of 95 °C for 3 s and 60 °C for 30 s. At the end, the relative expression level of target gene was determined as $2^{-\Delta\Delta CT}$

Statistical analysis

All of the quantification of the flow data was based on at least three analyses from at least three mice. All the data were presented as mean ± SD. Statistical significance was determined by a one-way ANOVA for multiple comparisons using Origin 8.5 software. $p < 0.05$ was considered statistically significant.

RESULTS

Body weight and epididymal adipose tissue weight

After feeding for 0–35 days, there was little difference in body weight gain between the Control Group and the Model Group. Body weight gains of the High Group and the Low Group were significantly lower than those of the Model Group. But when fed for 35–56 days, there was little difference in the body weight gain between the Control Group, the High Group, and the Low Group. The body weight gain of the Model Group was higher than those of the other three groups (Fig. 1a). The resulting epididymal adipose tissue weights and body weights are shown in Fig. 1b. The epididymal adipose tissue weights of the three high-fat diet groups were higher than the Control Group. The epididymal adipose tissue weights of the Low Group and the High Group were significantly lower than those of the Model Group ($p < 0.01$).

Serum lipid levels

As shown in Fig. 2a, the plasma TC concentrations in the Low Group and the High Group were signifi-

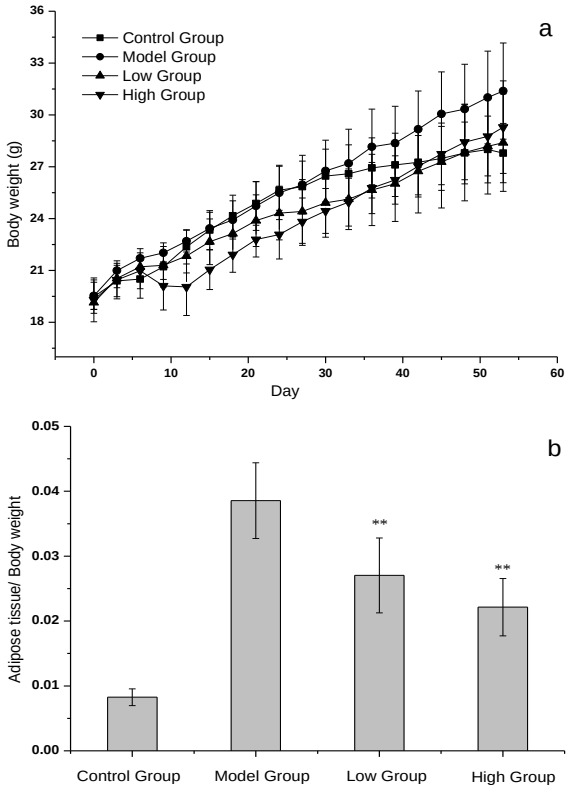

Fig. 1 Effects of dietary EMZAP on (a) body weight and (b) epididymal adipose tissue. Each value is mean ± SEM, $n = 10$ for each group; * $p < 0.05$ and ** $p < 0.01$ vs. Model Group.

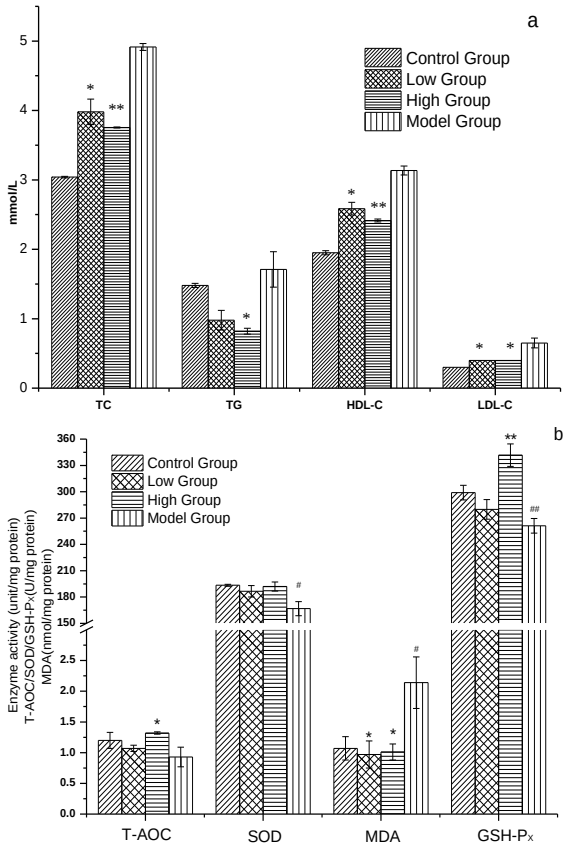

Fig. 2 Effects of EMZAP on (a) serum and (b) the antioxidant capacity of organisms in the liver. Each value is mean ± SEM, $n = 10$ for each group; * $p < 0.05$ and ** $p < 0.01$ vs. Model Group; # $p < 0.05$ and ## $p < 0.01$ vs. Control Group.

cantly lower than that of the Model Group ($p < 0.05$ and $p < 0.01$). The plasma TG concentration in the Low Group was slightly reduced compared with the Model Group, but the difference was not significant ($p > 0.05$); while the High Group showed a significant decrease compared to the Model Group ($p < 0.05$). The plasma HDL-C concentrations in the Low Group and the High Group were significantly lower than in the Model Group ($p < 0.05$ and $p < 0.01$). The plasma LDL-C concentrations in the Low Group and the High Group were significantly lower than in the Model Group ($p < 0.05$). In addition, the plasma levels of TC, TG, HDL-C, and LDL-C were lower in the High Group than in the Low Group.

Antioxidant capacity of organisms in liver

The effects of EMZAP treatment on antioxidant capacity of organisms in the liver are shown in Fig. 2b. T-AOC levels in the High Group and the Low Group were increased compared with that in the Model Group ($p < 0.05$ and $p > 0.05$). The T-AOC level in the High Group had little increase compared with the Model Group ($p > 0.05$). The SOD level was significantly lower in the Model Group compared to the Control Group ($p < 0.05$). The SOD levels in the High Group and the Low Group were increased compared with that in the Model Group ($p > 0.05$); and they were a little decreased compared with the Control Group ($p > 0.05$). The GSH-PX level was significantly lower in the Model Group compared to the Control Group ($p < 0.01$). The GSH-PX levels in the High Group and the Low Group were increased compared with that in the Model Group ($p < 0.01$ and $p > 0.05$). The GSH-PX level in the High Group had little increase compared with the Model Group ($p > 0.05$). The MDA level was significantly increased in the Model Group compared to the Control Group ($p < 0.05$). The MDA levels in the High Group and the Low Group were significantly

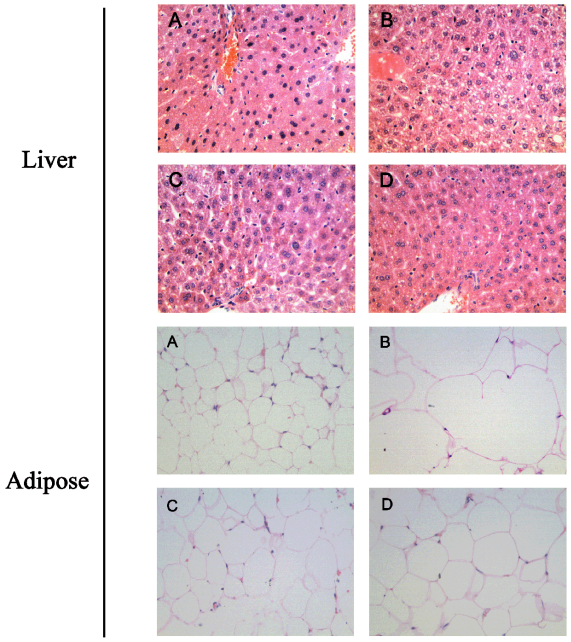

Fig. 3 Histological analyses of mouse liver and epididymal adipose tissues of each group. Samples were stained with ematoxylin and eosin, and were photographed at 400 × magnification. (A) Control Group, (B) Model Group, (C) High Group, and (D) Low Group.

lower compared with the Model Group ($p < 0.05$ and $p < 0.05$) and the Control Group ($p > 0.05$).

Histopathological changes in liver and epididymal adipose tissues

The liver plays a central role in controlling the homeostasis of the whole body, including lipid and carbohydrate metabolism. As shown in Fig. 3, there was extensive hepatocyte vacuolation in animals in the Model Group, which were fed with a high-fat diet, indicating fat accumulation in the tissue. However, EMZAP treatment efficiently ameliorated lipid accumulation in hepatocytes. Moreover, histological analysis of the epididymal adipose tissue, shown in Fig. 3, confirmed that the adipocyte size was markedly bigger in the Model Group than in the other groups. After 8 weeks of supplementation, the two dosages of EMZAP significantly attenuated adipocyte sizes to different degrees. Histological analyses of both liver and epididymal adipose tissues confirmed the improvement of EMZAP in high-fat-diet induced obesity models.

Gene expression in liver

According to the above results, EMZAP exert anti-obesity effects and cause improvement of cholesterol accumulation. Therefore, we proceeded to examine fat metabolism genes in the liver. As shown in Fig. 4, the expression of HMG-CoA-R was significantly decreased in the Low Group and the High Group when compared with the Model Group ($p > 0.05$ and $p < 0.01$). Furthermore, in the Low Group and the High Group, EMZAP decreased the expression of HMG-CoA-R compared with the Control Group ($p > 0.05$). In addition, the expression of CYP7A1 was significantly increased in the Low Group and the High Group as compared with the Model Group ($p < 0.05$) and the Control Group ($p > 0.05$). In the Model Group, the high-fat diet evidently reduced the expression of LDLR in liver tissues in comparison with the Control Group ($p < 0.01$). Also, in the Low Group and the High Group, EMZAP increased the expression of LDLR when compared with the Model Group ($p > 0.05$ and $p < 0.05$). Moreover, the expression of LXRα was significantly decreased in the Model Group when compared with the Control Group ($p < 0.05$). As well, the expression of LXRα showed no significant differences in the Low Group and the High Group when compared with the Model Group.

DISCUSSION

The male zooid of *Antheraea pernyi* is an animal-derived medicine in China. According to the Dictionary of Traditional Chinese Medicine, the major components of the male zooid are proteins, with more than 20 kinds of free amino acids, and cytochrome c [17].

In modern society, obesity and overweight status affect people of all ages and irrespective of the social class. Being treated as a "global epidemic", this calls for awareness and implementation of health policies to help promote healthy lifestyle and recognize the dangers of obesity [18]. Lyu et al [19] used mice as animal models to investigate the acute toxicity and mutagenicity of tussah male moth powder. The results indicated that tussah male moth powder had no acute toxicity, mutagenicity, or teratogenicity in mice. That study provided an experimental basis for toxicology studies and safety evaluations of medicine and food developed from male tussah moths. Jiang et al [20] reported that male China oak silkworm liquid played a significant role in the prevention of alcohol-induced liver fibrosis in mice; low-doses seemed to work more effec-

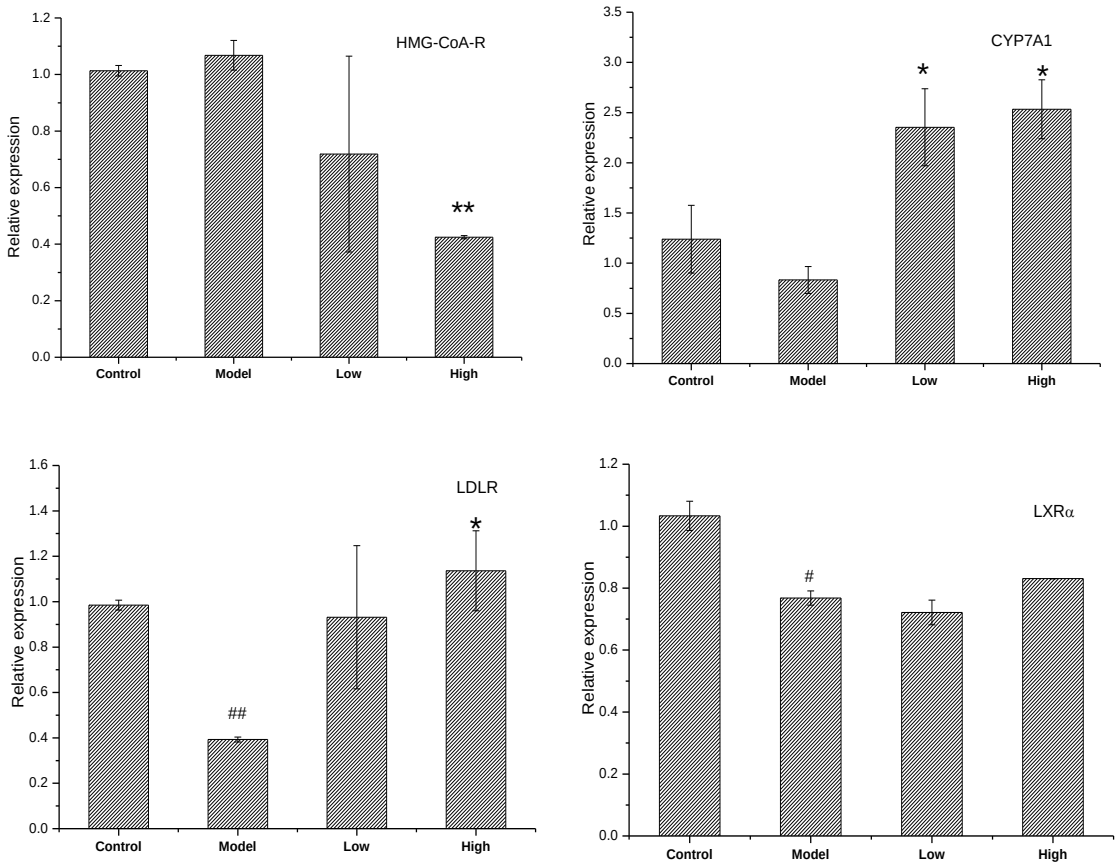

Fig. 4 Effects of EMZAP on gene expression in the liver. Each value is mean ± SEM, $n = 10$ for each group; $^{*}p < 0.05$ and $^{**}p < 0.01$ vs. Model Group; $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ vs. Control Group.

tively than high doses. In this paper, we found that EMZAP were able to alleviate high-fat-diet-induced obesity and reduce cholesterol accumulation. As shown in Fig. 1, the body weight of the Model Group was higher than that in the other three groups, which received EMZAP by gavage. In addition, the epididymal adipose tissue weights of the Low Group and the High Group were significantly lower than those of the Model Group ($p < 0.01$). The visual estimation of tissue entities under the microscope was the most direct and still the most investigative method used to determine the cellularity of a given tissue [21]. According to our histological analysis, the adipocyte sizes of the Low Group and the High Group were markedly smaller than that of the Model Group (Fig. 3). Our results showed that EMZAP can reduce fat accumulation in mice fed on high-fat diet.

The liver plays a major role in lipid metabolism, including lipid degradation to bile acids and elimination to bile. Most of the cholesterol that deposits in tissues must be transported to the liver by a process referred to as RCT [22]. HDL has been identified as taking a central role in the RCT pathway, although plasma levels of HDL-C are not necessary for RCT function. Low HDL-C levels, coexisting with elevated triglycerides and small-dense LDL particles, are characteristic of dyslipidemia in patients [23]. In our research, the plasma levels of TC, TG, HDL-C, and LDL-C were lower in the EMZAP-treated Groups than in the Model Group. In addition, the histological analysis of liver tissues showed that EMZAP treatment efficiently reduced lipid accumulation in hepatocytes. The antioxidant capacity of organisms is closely related to health, and it has two parts: enzymatic reactions and non-enzymatic reactions. The enzymatic reactions include those of SOD, CAT, GSH-PX, and others. Their function is to decompose free radicals and remove catalytic metal ions. MDA is one of the important products of membrane lipid peroxidation, so its level is an indicator of peroxidation in an organism. Oxidative stress is the impairment of

oxidant/antioxidant equilibrium causing molecular and cellular tissue damages [24]. EMZAP also enhanced the antioxidant capacity of liver in mice fed on high-fat diet. There was not much difference in the liver weights of mice in different groups (data not shown). However, histological analysis showed that EMZAP significantly reduced the accumulation of epididymal adipose tissue in the liver.

We examined the mRNA levels of the key regulatory genes in RCT (HMG-CoA-R, CYP7A1, LDLR, and LXRα) of mice. Previous studies had shown that a decreased expression of HMG-CoA-R is beneficial for suppressing the synthesis of hepatic cholesterol [25]. Our research also got the same results; the expression of HMG-CoA-R in the High Group was significantly decreased compared with the Model Group ($p < 0.01$). The expressions of CYP7A1and LDLR in the EMZAP-treated groups (the Low Group and the High Group) was increased compared with the Model Group. The increased expressions of these two genes were capable of reducing the cholesterol content in the plasma [26, 27]. The increased expression of LXRα has been shown to improve hyperlipidemia and prevent atherosclerosis [28]. However, in our paper, only the expression of LXRα in the High Group was slightly higher than that in the Model Group ($p > 0.05$), which indicated that EMZAP treatment had no obvious effect on the expression of LXRα.

CONCLUSION

In this paper, EMZAP were proved to be able to reduce body weight and serum lipid. The results of genes expression in the liver showed that EMZAP could improve the cholesterol accumulation. The histological results showed that EMZAP could improve the pathological changes of liver. In conclusion, EMZAP had good anti-obesity attributes and improvement in conditions arising out of cholesterol accumulation. All of these results are expected to provide a theoretical basis for the high-value utilization of tussah moths.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at http://dx.doi.org/10.2306/scienceasia1513-1874.2021.021.

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REFERENCES

1. Togo M, Konari N (2018) Effects of a high-fat diet on superoxide anion generation and membrane fluidity in liver mitochondria in rats. *J Int Soc Sport Nutr* **15**, ID 13.
2. Watanabe T, Hata K, Hiwatashi K, Hori K, Suzuki N, Itoh H (2010) Suppression of murine preadipocyte differentiation and reduction of visceral fat accumulation by a petasites japonicas ethanol extract in mice fed a high-fat diet. *Biosci Biotechnol Biochem* **74**, 499–503.
3. Kopelman PG (2000) Obesity as a medical problem. *Nature* **404**, 635–643.
4. Brouns F (2018) Overweight and diabetes prevention: is a low-carbohydrate-high-fat diet recommendable? *EurJ Nutr* **57**, 1301–1312.
5. Wu HL, Liu QZ, Kalavagunta PK, Huang QL, Lv WT, An XH, Chen HJ, Wang T, et al (2018) Normal diet vs. high fat diet – a comparative study: behavioral and neuroimmunological changes in adolescent male mice. *Metab Brain Dis* **33**, 177–190.
6. Tonkin A (2001) High-density lipoprotein cholesterol and treatment guidelines. *Am J Cardiol* **88**, 41–44.
7. Keshk WA, Noeman SA (2015) Impact of chicory-supplemented diet on HMG-CoA reductase, Acetyl-CoA carboxylase, visfatin and anti-oxidant status in triton WR-1339-induced hyperlipidemia. *J Food Biochem* **39**, 164–172.
8. Mbikay M, Mayne J, Sirois F, Fedoryak O, Raymond A, Noad J, Chrétien M (2018) Mice fed a high-cholesterol diet supplemented with quercetin-3-glucoside show attenuated hyperlipidemia and hyperinsulinemia associated with differential regulation of PCSK9 and LDLR in their liver and pancreas. *Mol Nutr Food Res* **62**, ID 1700729.
9. González-Torres L, Vázquez-Velasco M, Olivero-David R, Sara B, Benedí J, González RR, González-Muñoz MJ, Francisco J (2015) Glucomannan and glucomannan plus spirulina added to pork significantly block dietary cholesterol effects on lipoproteinemia, arylesterase activity, and CYP7A1 expression in Zucker fa/fa rats. *J Phys Biochem* **71**, 773–784.
10. Noratto GD, Lage NN, Chew BP, Mertens-Talcott SU, Talcott ST, Pedrosa ML (2018) Non-anthocyanin phenolics in cherry (*Prunus avium* L.) modulate IL-6, liver lipids and expression of PPARδ and LXRs in obese diabetic (db/db) mice. *Food Chem* **6**, ID 20.
11. Ming D, Li SZ (1981) *Compendium of Materia Medica*, 4th edn, The People's Health Press, Beijing.
12. Qu Z, Steiner H, Engstrom A, Bennich H, Boman HG (1982) Insect immunity: isolation and structure of cecropins B and D from pupae of the chinese oak silk moth, *Antheraea pernyi*. *Eur J Biochem* **127**, 219–224.
13. Li S, Zhang B, Zhang WD, Ma TH, Huang Y, Yi LH, Yu JM (2005) Immunization of mice with concentrated

liquor from male zooid of *Antheraea pernyi*. *World J Gastroenterol* **11**, 4254–4257.
14. Zhao WH, Li L, Zhang B, Zhang WD, Zong M, Tang JD, Zhang HY, Li S (2008) Enhanced CD^{4+} T cell activities and modulation of Th1/Th2 lineage development in irradiated rats due to treatment with the male zooid of *Antheraea pernyi* extracts. *World J Gastroenterol* **14**, 2094–2099.
15. Zou DQ, Zheng SX, Luan HJ, Yu ZC, Fan ZQ, Zhang WD (2006) The protecting effect on liver cell and therapeutic effects on hyperlipoproteinemia of the concentrated extract of tussah male moth. *Sci Sericult* **32**, 530–534.
16. Zou DQ, Fan ZQ, Li ZF, Guo G, Nie L, Song HY, Yu ZC (2012) Production process condition improvement and quality inspection of concentrated extract of tussah male moth. *Sci Sericult* **38**, 942–948.
17. Liu XM, Zhou LS (2003) The anti-fatigue function of the capsule wei li kang. *Guangdong Yiyao* **24**, 248–249.
18. Wang X, Liu F, Gao Y, Xue CH, Li RW, Tang QJ (2018) Transcriptome analysis revealed anti-obesity effects of the sodium alginate in high-fat diet-induced obese mice. *Int J Biol Macromol* **115**, 861–870.
19. Lyu JY, Zhang Y, Zhang GY, Zhang HD, Xue Q, Sun JH, Yang SF, Zhang XZ, et al (2016) Acute toxicity and mutagenicity test of tussah male moth powder on kunming mice. *Sci Sericult* **42**, 733–737.
20. Jiang YH, Zhang YY, Zhang WD, Jia Q, Wang ZP, Wang ZX, Wu LC (2016) Preventive effect and mechanism of male china oak silkworm liquid on the alcoholic liver fibrosis in mice. *Prog Mod Biomed* **16**, 2836–2841.
21. Greenstein JP (1954) *Biochemistry of Cancer*, Academic Press, New York, NY.
22. Guan JZ, Tamasawa N, Murakami H, Matsui J, Tanabe J, Matsuki K, Yamashita M, Suda T (2007) HMG-CoA reductase inhibitor, simvastatin improves reverse cholesterol transport in type 2 diabetic patients with hyperlipidemia. *J Athero Throm* **15**, 20–25.
23. Krauss RM (2004) Lipids and lipoprotein in patients with type 2 diabetes. *Diabetes Care* **27**, 1496–1504.
24. Yang RL, Shi YH, Hao G, Li W, Le GW (2008) Increasing oxidative stress with progressive hyperlipidemia in human: Relation between malondialdehyde and atherogenic index. *J Clin Biochem Nutr* **43**, 154–158.
25. Ren RD, Gong JJ, Zhao YY, Zhuang XY, Ye Y, Lin WT (2017) Sulfated polysaccharides from Enteromorpha prolifera suppress SREBP-2 and HMG-CoA reductase expression and attenuate non-alcoholic fatty liver disease induced by a high-fat diet. *Food Func* **8**, 1899–1904.
26. Yang Y, Liu H, Shi ZX, Wang LX, Cheng XZ, Wang SH, Ren GX (2014) Mung bean decreases plasma cholesterol by up-regulation of CYP7A1. *Plant Food Hum Nutr* **69**, 134–136.
27. Paththinige CS, Rajapakse JRDK, Constantine GR, Sem KP, Singaraja RR, Jayasekara RW, Dissanayake VHW (2018) Spectrum of low-density lipoprotein receptor (LDLR) mutations in a cohort of Sri Lankan patients with familial hypercholesterolemia – a preliminary report. *Lipids Health Dis* **17**, 1.
28. Benítez-Santana T, Hugo SE, Schlegel A (2017) Role of intestinal LXRα in regulating post-prandial lipid excursion and diet-induced hypercholesterolemia and hepatic lipid accumulation. *Front Physiol* **8**, 280.

Appendix A. Supplementary data

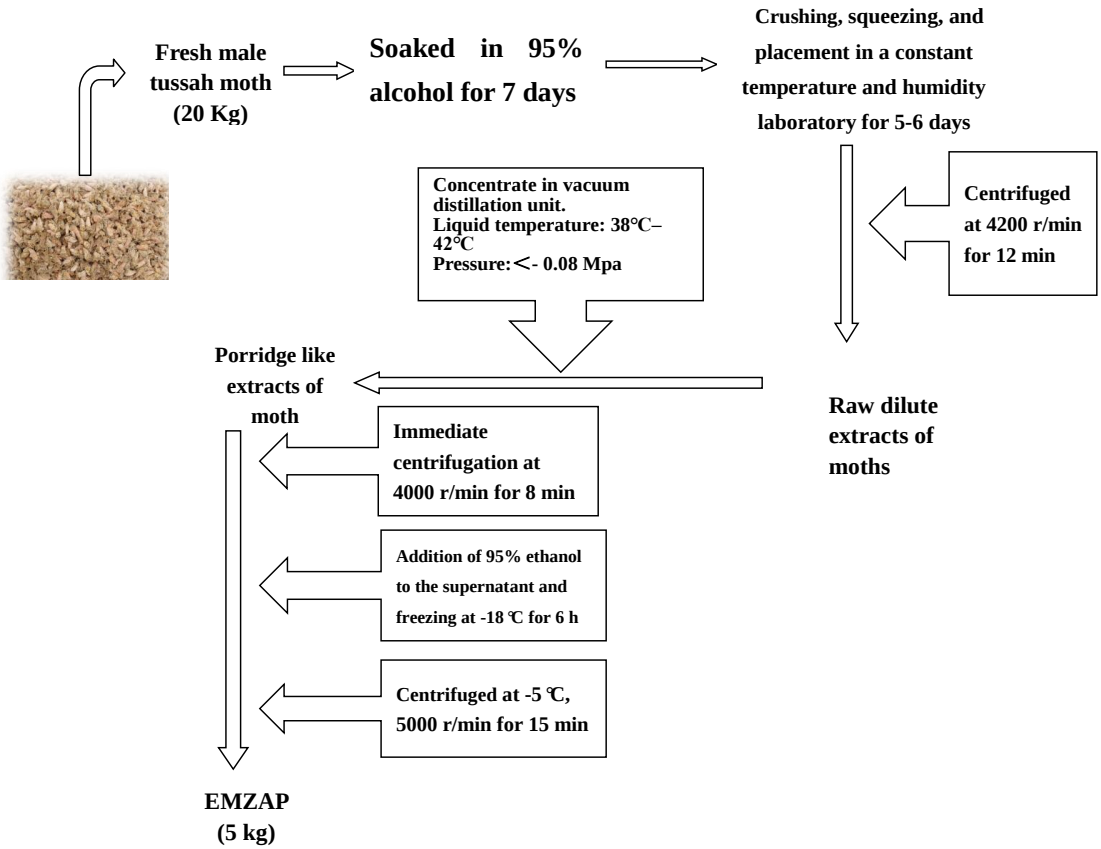

Fig. S1 Production process of EMZAP.