Effects of sanshoamides and capsaicinoids on plasma and liver lipid metabolism in hyperlipidemic rats

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Abstract The objective of this study was to determine the effects of sanshoamides and capsaicinoids on plasma and liver lipid levels and the mRNA expression levels of key receptors involved in cholesterol metabolism in hyperlipidemic rats. A total of 56 three-week-old female Sprague–Dawley rats were assigned to 7 treatment groups based on initial body weight ($n = 8$ rats per group). With certain combinations of sanshoamides and capsaicinoids significantly increased food intake, reduced lipid levels in blood and liver, improved histological characteristics of a fatty liver, down regulated mRNA expression levels of cholesterol 7- α -hydroxylase (CYP7A1), 3-hydroxyl-3-methylglutaryl CoA (HMG-CoA) and Farnesoid X Receptor (FXR) in liver and apical sodium-dependent bile acid transporter, Ileal Bile Acid Binding Protein and FXR in the ileum in hyperlipidemic rats. These results indicated that dietary supplementation with sanshoamides and capsaicinoids reduced blood lipid levels and improved cholesterol metabolism in hyperlipidemic rats.

Keywords Capsaicinoid · Cholesterol metabolism · Hyperlipidemic rat · Sanshoamide

Abbreviations

HMG-CoA 3-hydroxyl-3-methylglutaryl CoA
CYP7A1 Cholesterol 7 α -hydroxylase
FXR Farnesoid X Receptor
IBABP Ileal Bile Acid Binding Protein

ASBT Apical sodium-dependent bile acid transporter
LDL-C Low-density lipoprotein cholesterol
TC Total cholesterol
TG Total triglycerides
HDL-C High-density lipoprotein cholesterol

Introduction

Hyperlipidemia is a significant risk factor for cardiovascular diseases such as coronary heart disease and peripheral artery diseases and is highly modifiable through food intake (Shi et al., 2014). Increased serum levels of low-density lipoprotein (LDL), a key feature of hyperlipidemia, can accelerate atherosclerosis (Kishida et al., 2003). Therefore, the reduction of high dietary lipid levels has been advocated as an important approach in preventing and slowing the progression of atherosclerosis (Ahuja et al., 2006). The beneficial effects of certain specific dietary components on cholesterol metabolism, especially natural compounds such as capsaicin and curcumin, have received considerable research attention (Kwon et al., 2003; Lee et al., 2011; Liang et al., 2013).

Several phytochemicals that are present in traditional spices have been reported to have hypolipidemic effects because they modulate the expression of target genes involved in lipid and lipoprotein metabolism (Kwon et al., 2003; Negulesco et al., 1987). Among these, *Capsicum annuum* L. is an important spice that contains many nutrients, including vitamin A and B, bioflavonoid rutin, carotene, iron, calcium, and potassium (Kwon et al., 2003). The main active ingredient in *Capsicum annuum* is Capsaicin (8-methyl-*N*-vanillyl-6-nonenamide), which typically makes up 46–77% of the total active ingredients. In a

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previously study, it was shown that ingestion of capsaicin had a significant weight-loss effect by promoting the secretion of the neurotransmitters acetylcholine and norepinephrine, which increased oxygen and energy consumption (Inoue et al., 2007). In addition, it has been shown that dietary capsaicin can improve the body mass index (BMI) of obese patients by promoting the oxidation of body fat, enhancing energy release, and reducing the storage of fat (Lejeune et al., 2007). Snitker et al. (2009) reported that 6 mg of capsaicin per day is a safe level to consume and is effective in reducing abdominal fat. However, the genetic and biological mechanisms by which capsaicin influences the production of body fat and whether and how it affects blood lipid levels are unclear. Investigating the ability of capsaicin to regulate genes involved in blood cholesterol homeostasis may provide insight into these underlying mechanisms of action.

The genus *Zanthoxylum* (family: *Rutaceae*) is a group of ~ 250 plant species, which leaves contain various substances, including alkaloids, coumarin, lignin, amides (including sanshoamides), fatty acids, sterols, and flavonoids (Kwon et al., 2003). Several studies have shown beneficial health-related effects of ingested sanshoamides. These included functioning as an antiplatelet aggregation factor (Le Liu et al., 2007) as well as acting as a relaxing the toroidal muscle of the stomach (Liu et al., 2010) and the longitudinal muscle of the ileum (Dekaney et al., 2008). The two main constituents of sanshoamides are β -sanshol and neoherculin (Kempaiah and Srinivasan, 2002; 2004). Previous studies have demonstrated that these substances exhibit anaesthetic, excitement, bacteriostatic dehumidification, insecticidal, and analgesic properties (Chen et al., 1999). To elucidate how these compounds act on blood vessels in the context of hyperlipidemia need to be further elucidated (Zhang et al., 2017). Information on the effects of dietary supplementation with sanshoamides and capsaicinoids on plasma lipid levels or on the expression of genes involved in cholesterol metabolism is limited.

According to our previous study, dietary supplementation of sanshoamides at 3 mg/kg/day regulated lipid metabolism in rats (data not published). In addition, in our early trials, we demonstrated that lipid levels in plasma and liver decreased by supplementation of capsaicinoids at 9 mg/kg/day (data not published). So, our previous research has shown that sanshoamides and capsaicinoids have a beneficial effect in cholesterol metabolism respectively. However, we do not know whether the combination of sanshoamides and capsaicinoids will have a better/worse effect in cholesterol metabolism. Therefore, in this study, we aimed to investigate the effect of dietary supplementation with a mixture of sanshoamides and capsaicinoids on blood lipid levels and the expression of genes involved in cholesterol metabolism in rats, and to determine an optimal

dosage for achieving a reduction in blood lipid levels. We anticipated that the findings would increase the current understanding regarding the effects of the active ingredients on cholesterol metabolism in hyperlipidemia.

Materials and methods

Test materials

Sanshoamides, containing 982.63 g/kg of active ingredient, were obtained from Chongqing Tengxin Inc. (Chongqing, China). Capsaicinoids were purchased from the Henan Bisiotech Company (Henan, China) and contained 355.42 g/kg capsaicin and 592.74 g/kg dihydrocapsaicin. Basic solid rat diet was purchased from the Chongqing Tengxin Company (Chongqing, China).

Animals and diets

All experimental procedures were performed in accordance with the guidelines that were approved by the Southwest University Animal Care and Use Committee (Chongqing, China) (Permit No. 2017-01-0045). A total of 56 three-week-old female Sprague–Dawley (SD) rats were assigned to 7 treatment groups based on initial body weight ($n = 8$ rats per group). Experimental procedures were approved by the Institutional Animal Care and Research Advisory Committee of the Southwest University (Chongqing, China). A total of 56 three-week-old (90–110 g) female Sprague–Dawley (SD) rats were obtained from the Chongqing Tengxin Company (Chongqing, China). Animals were housed in an environment at an ambient temperature of 22 ± 2.0 °C and a relative humidity of 50%. The light/dark cycle was 12:12. Animals were acclimated for 1 week under these conditions and were fed a commercial solid diet (Chongqing Tengxin, Inc.). The composition of the commercial solid diet was as follows: corn starch, 548.5 g; mineral mixture, 36.0 g; casein, 190.0 g; vitamin mixture, 10.0 g; sucrose, 110.0 g; L-Cystine, 4.0 g; choline chloride, 1.5 g; and soybean oil, 100.0 g. Diet and water were offered ad libitum for a total of 28 days. Thereafter, the rats were divided into 7 treatment groups ($n = 8$ per group), based on body weight and housed in stainless steel screen-bottom cages. Body weight was recorded every 3 days and feed intake was recorded daily. During the experimental period of 21 days, rats were fed the commercial diet and were given daily oral supplementation of one of 7 mixtures, according to treatment, administered by gavage. The treatments were as follows: normal control group (2 mL/day normal saline); hyperlipidemia control group (2 mL/day lipid emulsion), group A (2 mL/day lipid emulsion and 3 mg/kg/day

sanshoamides), group B (2 mL/day lipid emulsion and 9 mg/kg/day capsaicinoids), group C (2 mL/day lipid emulsion, 1 mg/kg/day sanshoamides and 8 mg/kg/day capsaicinoids), group D (2 mL/day lipid emulsion, 2 mg/kg/day sanshoamides and 7 mg/kg/day capsaicinoids), and group E (2 mL/day lipid emulsion, 3 mg/kg/day sanshoamides and 6 mg/kg/day capsaicinoids). During the experiment, rats were given free access to food and water.

Lipid emulsion preparation

A total of 200 g of lard was melted in a 1000 mL beaker on a heated electric furnace (Being Technologies Corp, Shanghai, China), before adding 100 g cholesterol (Chongqing Tengxin Inc, Chongqing, China), 20 g sodium deoxycholic acid and 10 g methyl thiouracil. The mixture was stirred until completely dissolved, and then 200 mL Tween-80, 200 mL propylene glycol, and 3000 mL distilled water were added, stirring continuously. The resulting mixture was cooled to room temperature, transferred to a 1000 mL volumetric flask, and diluted with distilled water to 1000 mL volume. The emulsion was stored at 37 °C and further melted in a water bath prior to experimental use.

Sample collection

On day 28, all rats were euthanized by venous administration of sodium pentobarbital (30 mg/kg of body weight). The ileums were removed, and the digesta was flushed with 4% saline. Samples were immediately frozen in liquid nitrogen and stored at − 80 °C for mRNA determination. Additionally, the right lobe of the liver was extracted, washed with 4% saline, dried, weighed, and stored at − 80 °C for mRNA determination and evaluation of the lipid metabolism index. From each rat, a piece of the liver was removed and fixed in 4% (w/v) paraformaldehyde for analysis of liver morphology. Blood samples were collected by jugular exsanguinations using a blood collection tube that contained heparin as an anticoagulant. Plasma was isolated by centrifugation at 1400×g at 4 °C for 15 min, and stored at − 80 °C until further analysis.

Growth performance measurements

During the experimental period, rats and feed were weighed on a per pen basis every day. Feed intakes (FI), body weight gain (BWG), and the feed efficiency ratio (FCR) were calculated. The formula used for calculating feed efficiency ratio was as follows:

$$\text{Food efficiency}/(\text{g}/100\text{g}) = \frac{\text{Body weight gain}/\text{g}}{\text{Food intake}/\text{g}} \times 100$$

Plasma biochemical analysis

Plasma levels of total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein-cholesterol (HDL-C) were determined by using a diagnostic kit (Beihai biotechnology, Shanghai, China) and the indications were measured by an automatic biochemical analyzer (Hitachi, Guangdong, China).

Liver biochemical analysis

Lipid levels in the liver were determined using a gravimetric analysis according as described by Zhang et al. (2013a; 2013b). The concentrations of TG and TC in liver were determined using commercial diagnostic kits (Beihai biotechnology, Shanghai, China).

Liver morphology analysis

Fixed liver samples were dehydrated and embedded in paraffin, cut at 3 µm thickness a microtome (Microtome Leica EG 1150H, Wetzlar, Germany), stained with hematoxylin and eosin, and evaluated for histological morphology. Images were taken at 40 × magnifications using a high-resolution digital camera (Nikon H550L, Tokyo, Japan).

Gene expression analysis in the liver and ileum

Total RNA was extracted from liver and ileum samples using Trizol reagent, according to the method described by Nagy et al. (2006). Extracted RNA was quantified by spectrophotometry by measuring absorbance at OD 260 nm. The purity of the isolated total RNA was assessed by measuring the ratio of absorbance at 260 and 280 nm. Purified mRNA (~ 1 µg) was used for cDNA synthesis using reverse transcriptase (Takara Biotechnology (Dalian) Co., Ltd., Dalian, China) in accordance with the manufacturer's instructions. The expression levels of target genes were determined by real-time PCR (RT-PCR) using a Light Cycler (Roche Diagnostics, Mannheim, Germany). In brief, cDNA (2 µl) was amplified in a total volume of 20 µl using specific primers (0.4 µm of each primer) and SYBR Premix Ex TaqII (Takara Biotechnology Co., Ltd., Dalian, China). Primers were obtained from Sangon Biological Engineering, Shanghai, China. The following primers were used:

CYP7A1 (sense, 5'-GAGGGATTGAAGCACAAGA ACC-3'; antisense, 5'-ATGCCAGAGAATAGCGAGGT-3'), HMG-CoA R (sense, 5'-GACCAACCTTCTACCT-CAGCAAG-3'; antisense, 5'-ACAACCTACCAGCCAT-CACAGT-3'), IBABP (sense, 5'-CAGACTTCCCCAAC

TATCACCAG-3'; antisense, 5'-TCAAGCCACCCTCTTG CTTAC-3'), ASBT (sense, 5'-GTGACATGGACCTCA GTGTTAGC-3'; antisense, 5'-GTAGGGGATCACAATC GTTCCT-3'), FXR (sense, 5'-ATAGCTTGGTCGTGGA GGTCAC-3'; antisense, 5'-GCTAAGGAAGTGCAGA-GAGAGG-3').

The PCR program involved an initial denaturation step for 30 s at 95 °C, an amplification step (40 cycles of 5 s at 95 °C), an annealing and extension step (40 cycles of 20 s at 60 °C), and a final extension step for 10 min 72 °C. Relative gene expression was calculated by using the crossing point of each target gene. The β -actin gene was used as a reference.

Statistical analysis

Data were subjected to the Levene's test for homogeneity of variances before further statistical analysis, and expressed as the mean $\pm$ SD. The data were analyzed by one-way ANOVA using SPSS statistical software (version 17.0, SPSS Inc, Chicago, IL, USA). Duncan's Multiple Range Test was used to identify differences between treatment groups and to separate mean values. Differences were considered statistically significant when $p < 0.05$.

Results and discussion

Growth performance

The effects of the treatments on growth performance are presented in Table 1. Compared with the normal (saline)

control group, the hyperlipidemic control group exhibited significantly increased body weight gain and decreased food intake ($p < 0.05$), however no effects of supplementation with sanshoamides and/or capsaicinoids on were observed. Food intake was reduced in hyperlipidemic control rats when compared to the normal control group. Rats in experimental group D exhibited significantly increased food intake compared with rats in the hyperlipidemic control group ($p < 0.05$), however there no significant differences were found between other experimental groups. In addition, no statistically significant differences were observed in feed efficiency of rats in the experimental groups compared with rats in the normal control groups, however mean values in all experimental groups (groups A, B, C, D, and E) tended to be reduced.

The hyperlipidemic rat model is the most common animal model for studying the cholesterol metabolism. Body weight gain was significantly increased by feeding lipid emulsion, which was similar with the results described in a previous study (Shi et al., 2014). These growth performance data suggested that lipid emulsion caused a hormonal imbalance in the hyperlipidemic rat. However, capsaicinoids and sanshoamides did not favorably modify the body weight gain and food intake. This absence on the effect on body weight gain and food intake was consistent with the findings of other studies in which diets supplemented with capsaicinoids and sanshoamides did not affect animal performance (Kempaiah and Srinivasan, 2006; Kim and Kim, 2010). The fact that we did not observe significant differences on body weight gain, and feed intake may be partly due to the lower levels of capsaicinoids and

Table 1 Effects of dietary treatment on body weight gain, total feed intake and feed efficiency of rats from day 1 to day 28^{1,2}

Treatment	Day 1 to day 28		
	Body weight gain (g)	Food intake (g)	Feed efficiency ratio (g/100 g)
Normal	68.75 $\pm$ 5.41*	460.58 $\pm$ 41.48*	15.76 $\pm$ 3.54
Hyperlipidemia	77.20 $\pm$ 6.12	404.65 $\pm$ 9.66 ^a	18.50 $\pm$ 2.14
A	75.47 $\pm$ 5.20	417.51 $\pm$ 35.54 ^{ab}	18.08 $\pm$ 3.57
B	74.69 $\pm$ 7.03	430.16 $\pm$ 41.26 ^{ab}	17.36 $\pm$ 1.86
C	76.14 $\pm$ 5.21	421.28 $\pm$ 12.56 ^a	16.84 $\pm$ 2.32
D	74.50 $\pm$ 6.38	448.48 $\pm$ 16.61 ^b	15.98 $\pm$ 2.51
E	73.60 $\pm$ 6.40	422.36 $\pm$ 21.83 ^a	17.95 $\pm$ 1.77

¹Rats were fed different doses of sanshoamides and capsaicinoids for 28 days. Results are expressed as the mean $\pm$ SD ($n = 8$ per treatment group). Means bearing different superscript letters are significantly different ($p < 0.05$)

²Normal, normal control group fed with basal diet; Hyperlipidemia, hyperlipidemia group fed with 2 mL/day lipid emulsion; A, Group A fed with 2 mL/day lipid emulsion and 3 mg/kg/day sanshoamides; B, Group B fed with 2 mL/day lipid emulsion and 9 mg/kg/day capsaicinoids; C, Group C fed with 2 mL/day lipid emulsion, 1 mg/kg/day sanshoamides and 8 mg/kg/day capsaicinoids; D, Group D fed with 2 mL/day lipid emulsion, 2 mg/kg/day sanshoamides and 7 mg/kg/day capsaicinoids; E, Group E fed with 2 mL/day lipid emulsion, 3 mg/kg/day sanshoamides and 6 mg/kg/day capsaicinoids

*Means are significantly different from the hyperlipidemia control group ($p < 0.05$)

sanshoamides as well as the shorter feeding duration compared to other studies (Zhang et al., 2013a; 2013b).

Plasma and liver lipid levels

Compared with the normal control group, hyperlipidemia group rats exhibited higher plasma concentrations of TC and TG ($p < 0.05$) and lower concentrations of HDL-C ($p < 0.05$). In groups A, B, C, D, and E, plasma TC concentrations were significantly decreased compared with the hyperlipidemia control group ($p < 0.05$) but the concentrations of HDL-C showed no such differences. Plasma concentrations of TG were significantly reduced in rats in experimental groups B, D, and E compared with the hyperlipidemia group ($p < 0.05$). Plasma concentrations of LDL-C were significantly reduced in the experimental groups B and E compared with rats in the hyperlipidemia group ($p < 0.05$, Fig. 1).

The lipid content of the liver of rats in the experimental groups (A, B, C, D, and E) showed a reducing trend versus the hyperlipidemia control group, and the lipid content of the liver was significantly reduced in rats in experimental groups A and B when compared with rats in the hyperlipidemia control group ($p < 0.05$). The concentrations of total cholesterol and TG in the liver were significantly higher in the hyperlipidemia control group compared with the normal control group ($p < 0.05$). However, TC concentrations in the liver were significantly reduced in experimental groups A, D, and E, compared with the hyperlipidemia control group ($p < 0.05$). Moreover, rats in experimental group E exhibited significantly decreased liver triglyceride concentrations compared with the hyperlipidemia control group ($p < 0.05$, Fig. 2).

The rat is the most common mammalian model used in studies of atherosclerosis and cardiovascular disease (Shi et al., 2014). Dyslipidemia is a metabolic disorder that constitutes a crucial risk factor for atherosclerosis and cardiovascular disease (Shi et al., 2014). LDL-C has been found to be the strongest risk factor among all serum lipids for atherosclerosis, because of the increased penetration of oxidated LDL-C into arterial walls and the ready deposition of excess LDL-C into the blood vessel walls, which is a major component of atherosclerotic plaques (Gotto and Brinton, 2004). In contrast, HDL, a lipoprotein that is responsible for the transport of blood cholesterol, plays an essential role in preventing atherosclerosis and cardiovascular events (Padmapriyadarsini et al., 2017). In particular, HDL-C plays a critical role in the reduction of cholesterol levels in the blood and peripheral tissues (Liu et al., 2010). In the present study, serum LDL-C levels as well as serum TC and TG levels, were significantly increased in hyperlipidemic rats when compared with healthy rats, and in the liver, increases in lipid levels as well as in TC and TG

Fig. 1 Effects of dietary treatment on plasma lipid levels in rats on day 28. Rats were fed different doses of sanshoamides and capsaicinoids for 28 days. Results are expressed as the mean $\pm$ SD, ($n = 8$ per treatment group). Means bearing different superscript letters are significantly different ($p < 0.05$). *Means are significantly different ($p < 0.05$) from the hyperlipidemia control group. Normal, normal control group fed with basal diet; Hyperlipidemia, hyperlipidemia group fed with 2 mL/day lipid emulsion; A, Group A fed with 2 mL/day lipid emulsion and 3 mg/kg/day sanshoamides; B, Group B fed with 2 mL/day lipid emulsion and 9 mg/kg/day capsaicinoids; C, Group C fed with 2 mL/day lipid emulsion, 1 mg/kg/day sanshoamides and 8 mg/kg/day capsaicinoids; D, Group D fed with 2 mL/day lipid emulsion, 2 mg/kg/day sanshoamides and 7 mg/kg/day capsaicinoids; E, Group E fed with 2 mL/day lipid emulsion, 3 mg/kg/day sanshoamides and 6 mg/kg/day capsaicinoids

concentrations were evident. These results were consistent with those of previous studies (Shi et al., 2014; Sun et al., 2017; Zhang et al., 2017). In addition, in the present study, there was some evidence that dietary supplementation with capsaicinoids and sanshoamides at certain dose combinations favorably modified the serum lipoprotein profile in hyperlipidemic rats by decreasing plasma TC, TG, and LDL-C levels and by reducing the liver concentrations of TC, hepatic lipids, and TG. The results indicated that the accumulation of hepatic fat-droplets was to some extent ameliorated by dietary supplementation with the capsaicinoids and sanshoamides in the hyperlipidemic rats, and that capsaicinoids and sanshoamides may have lipid-lowering effects. These findings were consistent with previous studies that have reported cholesterol-lowering effects of capsaicinoids on ovariectomized rats (Zhang et al., 2013a; 2013b). Whilst previous studies have demonstrated similar serum and/or liver cholesterol and lipid lowering effects of capsaicinoids and red pepper (Kwon et al., 2003; Liang et al., 2013), the mechanisms behind these effects remain unknown. In particular, it is not known whether key genes involved in cholesterol metabolism are involved. In the present study, we aimed to address this by investigating the expression of the following key genes involved in cholesterol metabolism: CYP7A1, HMG-CoA, and FXR in the liver and ASBT, IBABP and FXR in the ileum.

Hepatic morphology

The structure of the liver tissue from subjects in the normal control group was intact and regular, with an even distribution of liver cells (Fig. 3). As expected; many lipid droplets were visible in liver cells of the hyperlipidemia control group. In addition, several lipid droplets were visible in liver cells of treatment groups A and B, but their abundance appeared to be reduced compared with the hyperlipidemia control group. The histology of the liver from rats in treatment groups C, D, and E was improved

compared with rats in the hyperlipidemia group and nearly approached that of the normal control group. These observations indicated that dietary supplementation with sanshoamides and capsaicinoids may have reduced the adverse effects of hyperlipidemia at a histological level in hyperlipidemic rats.

Fig. 2 Effects of dietary treatment on liver lipid levels in rats on day 28. Rats were fed different doses of sanshoamides and capsaicinoids for 28 days. Results are expressed as the mean $\pm$ SD, ($n = 8$ per treatment group). Means bearing different superscript letters are significantly different ($p < 0.05$). *Means are significantly different ($p < 0.05$) from the hyperlipidemia control group. Normal, normal control group fed with basal diet; Hyperlipidemia, hyperlipidemia group fed with 2 mL/day lipid emulsion; A, Group A fed with 2 mL/day lipid emulsion and 3 mg/kg/day sanshoamides; B, Group B fed with 2 mL/day lipid emulsion and 9 mg/kg/day capsaicinoids; C, Group C fed with 2 mL/day lipid emulsion, 1 mg/kg/day sanshoamides and 8 mg/kg/day capsaicinoids; D, Group D fed with 2 mL/day lipid emulsion, 2 mg/kg/day sanshoamides and 7 mg/kg/day capsaicinoids; E, Group E fed with 2 mL/day lipid emulsion, 3 mg/kg/day sanshoamides and 6 mg/kg/day capsaicinoids

Expression of genes associated with cholesterol metabolism in the ileum and liver

The mRNA levels of HMG-CoA and FXR in the livers of rats in the hyperlipidemia control group were higher compared to those in the normal control group ($p < 0.05$), whereas the mRNA levels of CYP7A1 were lower ($p < 0.05$) (Fig. 4). In the ileum, mRNA levels of ASBT and IBABP were higher in the hyperlipidemic group compared with the normal control group, while mRNA levels of FXR were lower ($p < 0.05$) (Fig. 5). Moreover, when compared with the hyperlipidemia group, rats in treatment groups C, D, and E exhibited increased

expression of CYP7A1 and FXR in the liver ($p < 0.05$) (Fig. 4) and reduced expression of ASBT in the ileum ($p < 0.05$) (Fig. 5). Furthermore, mRNA levels of HMG-CoA reductase in the liver were reduced in treatment group E ($p < 0.05$), whereas those of IBABP were reduced in both groups C and E, and those of FXR in the ileum were increased in groups D and E ($p < 0.05$).

The liver is the main organ for endogenous cholesterol synthesis in both the rat and human, in which 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase is the rate-limiting enzyme (Zhang et al., 2013a; 2013b). This enzyme facilitates the initiation of cholesterol synthesis by catalyzing the synthesis of mevalonate (MVA) from HMG-CoA, and further generating TC via activation squalene (Xu et al., 2016). In previous reports, it has been suggested that levels of HMG-CoA reductase mRNA and protein were unaffected by the ingestion of capsaicinoids in ovariectomized rats (Zhang et al., 2013a; 2013b). However, Xu et al. (2016) showed that alisol acetates, the main active ingredients of the traditional Chinese medicine *Alismatisrhizoma*, lowered TC levels via inhibiting the activity of HMG-CoA reductase, possibly by directly and competitive binding with HMG-CoA. In the present study, we found that mRNA levels of HMG-CoA reductase were raised in hyperlipidemic rats, and that dietary supplementation with sanshoamides and capsaicinoids reduced this effect. The mechanism of such an effect is unknown but

Fig. 3 Histology of the livers of female Sprague-Dawley rats on day 28 (at 20 times magnification). Liver sections were stained with hematoxylin-and-eosin. Control, normal control group fed with basal diet; Hyperlipidemia, hyperlipidemia group fed with 2 mL/day lipid emulsion; A, Group A fed with 2 mL/day lipid emulsion and 3 mg/kg/day sanshoamides; B, Group B fed with 2 mL/day lipid emulsion

and 9 mg/kg/day capsaicinoids; C, Group C fed with 2 mL/day lipid emulsion, 1 mg/kg/day sanshoamides and 8 mg/kg/day capsaicinoids; D, Group D fed with 2 mL/day lipid emulsion, 2 mg/kg/day sanshoamides and 7 mg/kg/day capsaicinoids; E, Group E fed with 2 mL/day lipid emulsion, 3 mg/kg/day sanshoamides and 6 mg/kg/day capsaicinoids

Fig. 4 Effects of dietary treatment on the relative mRNA expression levels of CYP7A1, HMG-CoA and FXR in the liver. Rats were fed with different doses of sanshoamides and capsaicinoids for 28 days. Results are expressed as mean $\pm$ SD, with $n = 8$ per treatment group. Means bearing different superscript letters are significantly different ($p < 0.05$). *Means are significantly different ($p < 0.05$) from the hyperlipidemia control group. Normal, normal control group fed with basal diet; Hyperlipidemia, hyperlipidemia group fed with 2 mL/day lipid emulsion; A, Group A fed with 2 mL/day lipid emulsion and 3 mg/kg/day sanshoamides; B, Group B fed with 2 mL/day lipid emulsion and 9 mg/kg/day capsaicinoids; C, Group C fed with 2 mL/day lipid emulsion, 1 mg/kg/day sanshoamides and 8 mg/kg/day capsaicinoids; D, Group D fed with 2 mL/day lipid emulsion, 2 mg/kg/day sanshoamides and 7 mg/kg/day capsaicinoids; E, Group E fed with 2 mL/day lipid emulsion, 3 mg/kg/day sanshoamides and 6 mg/kg/day capsaicinoids

may involve binding of the active ingredients to HMG-CoA reductase, as observed by Xu et al., (2016).

FXR is activated by bile acids and other cholesterol derivatives that regulate bile acid and cholesterol homeostasis and has also been shown to play a role in insulin homeostasis (Unsworth et al., 2017). It is mainly expressed in the liver, intestine, kidney, and adrenal glands (Kim et al., 2016). In the liver, FXR inhibits bile acid synthesis through the induction of a small heterodimer partner in a negative feedback loop (Mencarelli et al., 2013). In the intestine, FXR inhibits the absorption of bile salts through the modulation of several transport proteins (Mencarelli et al., 2013). The results of the present study showed that levels of FXR mRNA were upregulated in the liver of hyperlipidemic rats, but down regulated in hyperlipidemic rats whose diets had been supplemented with sanshoamides and capsaicinoids. Conversely, in the ileum, levels of FXR mRNA were downregulated in hyperlipidemic rats but increased in rats that were given a diet that had been supplemented with sanshoamides and capsaicinoids. This downregulation of FXR in the liver and upregulation in the ileum indicated that dietary supplementation with sanshoamides and capsaicinoids improved obesity and

Fig. 5 Effects of dietary treatment on the relative mRNA expression levels of ASBT, IBABP, FXR in the ileum. Rats were fed with different doses of sanshoamides and capsaicinoids for 28 days. Results are expressed as mean $\pm$ SD, with $n = 8$ per treatment group. Means bearing different superscript letters are significantly different ($p < 0.05$). *Means are significantly different ($p < 0.05$) from the hyperlipidemia control group, Normal, normal control group fed with basal diet; Hyperlipidemia, hyperlipidemia group fed with 2 mL/day lipid emulsion; A, Group A fed with 2 mL/day lipid emulsion and 3 mg/kg/day sanshoamides; B, Group B fed with 2 mL/day lipid emulsion and 9 mg/kg/day capsaicinoids; C, Group C fed with 2 mL/day lipid emulsion, 1 mg/kg/day sanshoamides and 8 mg/kg/day capsaicinoids; D, Group D fed with 2 mL/day lipid emulsion, 2 mg/kg/day sanshoamides and 7 mg/kg/day capsaicinoids; E, Group E fed with 2 mL/day lipid emulsion, 3 mg/kg/day sanshoamides and 6 mg/kg/day capsaicinoids

metabolic syndrome in hyperlipidemia rats and was consistent with the findings described by others (Dekanay et al., 2008; Gu et al., 2016).

Cholesterol 7 α -hydroxylase (CYP7A1) is a cytochrome P450 enzyme and is the rate-limiting enzyme in the synthesis of bile acid from cholesterol (Yun et al., 2016). In rodents, more than 75% of the conversion of cholesterol into bile acids is regulated by CYP7A1, in humans, this ratio is much higher (about 90%) (Zhang et al., 2013a; 2013b). It is regulated via a negative feedback mechanism and is suppressed by high bile acid activity (Zhang et al., 2013a; 2013b). Bile acids that are recycled from the intestine activate FXR, thereby leading to the induction of an orphan nuclear receptor called the small heterodimer partner (SHP). Together, these interact with Fetoprotein Transcription Factor (FTF) to inhibit the trans-activation of the CYP7A1 gene (Liu et al., 2016). In the present study, CYP7A1 mRNA levels were downregulated in the livers of hyperlipidemic rats when compared with rats in the normal control group, but dietary supplementation with capsaicinoids and sanshoamides increased their levels compared with the hyperlipidemia control group. These results were consistent with the findings described by Liang et al.

(2013) who reported elevated CYP7A1 levels in the livers of rats fed a high-cholesterol diet and suggested that this stimulated the conversion of cholesterol into bile acids. It therefore seems plausible that the increased expression of CYP7A1 observed in the present study may represent part of the mechanism by which capsaicinoids and sanshoamides reduced cholesterol levels in the hyperlipidemic rats fed lipid diets.

Bile acids are the end products of cholesterol metabolism, and plasma cholesterol can be lowered by disrupting bile acid reabsorption (Zhang et al., 2013a; 2013b). Intestinal absorption of bile salts is mediated significantly by the ASBT transporter present in brush-border membranes and IBABP in epithelial cells of the intestinal villus (Zhang et al., 2013a; 2013b). The present study was the first to report on the effects of capsaicinoids and sanshoamides on the gene expressions of ileal ASBT and IBABP in hyperlipidemic rats. In addition, in the previous study, we showed that dietary capsaicinoids did not affect IBABP or ASBT mRNA levels or protein expression in the ilea of ovariectomized rats, but significantly increased total bile acids in the feces (Zhang et al., 2013a; 2013b). In another study, it was shown that, in the ileum, mRNA levels of ASBT and IBABP were markedly up regulated in capsaicinoid supplemented ovariectomized rats, and that levels of bile acids in the feces were concurrently lowered (Zhang et al., 2013a; 2013b). In this study, we showed that the mRNA levels of these two genes in ileum were upregulated in hyperlipidemic rats compared with a normal control group, but were downregulated in hyperlipidemic rats whose diets had been supplemented with capsaicinoids and sanshoamides. In addition, capsaicinoids and sanshoamides significantly upregulated FXR mRNA levels in ileum. These data suggested that, in the rat, dietary capsaicinoids and sanshoamides do not interrupt or promote the enterohepatic circulation of bile acids but can enhance fecal bile acid excretion. The increased excretion of bile acids may have increased the demand for cholesterol for the *de novo* synthesis of bile acids, thereby decreasing the level of cholesterol, as was put forward by others (De Smet et al., 1995; Zhang et al., 2013a; 2013b). Therefore, we proposed that the observed reductions in cholesterol levels in the serum and livers of hyperlipidemic rats whose diets were with capsaicinoids and sanshoamides were mediated by changes in the expression of key genes involved in cholesterol metabolism. In conclusion, these results suggested that dietary supplementation with sanshoamides and capsaicinoids prevented the dysregulation of cholesterol metabolism in rats.

In conclusion, in this study, we demonstrated that dietary supplementation with certain combination levels of sanshoamides and capsaicinoids significantly increased food intake, reduced lipid levels in the blood and liver,

improved histological symptoms of a fatty liver, and improved cholesterol metabolism in hyperlipidemic rats. In addition, dietary supplemented groups exhibited down regulated levels of HMG-CoA and FXR mRNA in the liver, and ASBT and IBABP in the ileum, and upregulated levels of FXR mRNA in the ileum. The ratio of sanshoamides to capsaicinoids that produced the greatest and most consistent effect was 3:6 (Group E). Collectively, our results suggested that dietary supplementation with sanshoamides and capsaicinoids reduced blood lipid levels and improved cholesterol metabolism in hyperlipidemic rats.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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