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Comparison of protective effects of hesperetin and pectolinarigenin on high-fat diet-induced hyperlipidemia and hepatic steatosis in Golden Syrian hamsters

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Abstract: A comparative study was conducted to determine whether hesperetin and pectolinarigenin could lower total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL), and high-density lipoprotein cholesterol (HDL) in a high-fat diet (HFD)-induced high lipid model in Golden Syrian hamsters. 48 Golden Syrian hamsters (8 weeks old) were fed with a HFD for 15 days. HFD induced significant increases in plasma TC, TG, LDL, and HDL. Then, these high lipid hamsters were divided into four groups and were administered with 0.5% sodium carboxymethyl cellulose (CMC-Na), hesperetin (100 mg/kg/day), pectolinarigenin (100 mg/kg/day) or atorvastatin (1.0 mg/kg/day), for 7 weeks. It was found that pectolinarigenin treatment resulted in significant reductions in body weight, adiposity index, serum levels of TC, TG and hepatic TC, TG and free fatty acid compared to those in control hamsters with hyperlipidemia (P<0.05). However, hesperetin treatment only caused reductions in plasma TC and hepatic TG levels. Besides, the hamsters treated with pectolinarigenin showed a relatively normal hepatic architecture compared to the hepatic steatosis shown in the control group. Moreover, the expressions of fatty-acid synthase (Fasn) and solute carrier family 27 member 1 (Slc27a1) involved in lipid biosynthesis, were suppressed in the pectolinarigenin-treated groups, and the expression of carnitine palmitoyltransferase 1A (Cpt1a) involved in fatty acid oxidation was increased in the pectolinarigenin-treated group. Taken together, these results suggest pectolinarigenin exerts stronger protective effects against hyperlipidemia and hepatic steatosis than hesperetin, which may involve the inhibition of lipid uptake and biosynthesis.
Key words: hesperetin, high-fat diet, lipid biosynthesis, pectolinarigenin

Introduction

Lipid homeostasis disruption is the main cause of metabolic diseases such as dyslipidemia, fatty liver, cardiovascular disease, atherosclerosis, type II diabetes, etc [1]. The major risk factors for metabolic diseases are higher levels of plasma cholesterol and triglycerides (TG and TC), low-density lipoprotein (LDL), lower levels of high-density lipoprotein (HDL), and other serum lipid abnormalities.

Pectolinarigenin is the main flavonoid compound and is present in *Linaria vulgaris* and some species of citrus fruits [2]. In our previous study, we found that flavonoid extracts present in the *Linaria vulgaris* attenuated hyperlipidemia and hepatic steatosis induced by a western-type diet [3]. In our more recent study, pectolinarigenin was found to affect the subcellular localization of sterol regulatory element-binding protein (SREBP) cleavage-activating protein (SCAP), thereby weakening the normal function of SREBP and reducing lipid accumulation induced by oleic acid [4]. Besides, compounds isolated from *Chromolaena odorata* leaves, whose structures are similar to pectolinarigenin, can inhibit lipogenesis [5]. Thus, pectolinarigenin maybe is capable of attenuating

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hyperlipidemia in vivo. Hesperetin is a flavonoid found in some citrus juices. It has been reported to reduce cholesteryl ester mass and inhibit apoB secretion by up to 74% [6]. Furthermore, it was found that hesperetin and its metabolites both showed plasma lipid-lowering activities evidenced by the decreased 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and cholesterol acyltransferase (ACAT) activities [7]. However, some questions need to be clarified: (1) Whether pectolinarigenin shows lipid-lowering effects *in vivo*? (2) How does pectolinarigenin show lipid-lowering effects compared with hesperetin?

Considering the different characteristics of hyperlipidemia between mice and human, rodents such as mice and rats are not easy to develop high-lipid animal models [8]. By contrast, Golden Syrian hamsters are easy to develop a hyperlipidemia model when fed a high-fat diet (HFD), and this model is more close to hyperlipidemia in human [9]. Besides, Golden Syrian hamsters are highly susceptible to the HFD which promotes quick and robust hyperlipidemia [10]. Thus, in this study, a high lipid model was developed in Golden Syrian hamsters and the effects of hesperetin and pectolinarigenin on hyperlipidemia and hepatic steatosis induced by the HFD were investigated. Atorvastatin treatment was used as a positive control in this study. To the best of our knowledge, this is the first comparative study that shows how hesperetin and pectolinarigenin ameliorated hyperlipidemia and hepatic steatosis in hamsters.

Materials and Methods

Chemicals and animals

Male Golden Syrian hamsters weighing 129–145 g were obtained from Vital River Laboratory (Animal Technology Co., Ltd., Beijing, China). All 48 animals were housed in three per cage, with free access to standard food and water, in a controlled environment (temperature 22 ± 2°C, humidity 50 ± 10%), under a 12 h light/12 h dark cycle and with access to high-fat diet food and water *ad libitum*. The animals were acclimatized and habituated to the laboratory for at least a week before being subjected to tests. The protocol was approved by the Institutional Animal Care and Use Committee of Jiangxi Science and Technology Normal University (No. 2013-012). Hesperetin and pectolinarigenin were purchased from OKA Biotechnology Co., Ltd. (Beijing, China). Atorvastatin (calcium salt, >98% purity) was bought from Meilunbio Co., Ltd. (Dalian, China). All antibodies were ordered and bought from a commercial company (Boster Biological Technology, Co., Ltd., Wuhan, China).

Experimental design

The whole experimental design and doses used in this study are described in Fig. 1. Briefly, 48 hamsters were fed HFD for 15 days to establish a hyperlipidemia model, the body weights were measured and blood was collected from the orbital sinus for biochemical parameter measurement. A HFD containing 62.5% powdered chow 5001, 35% laerd, 2% cholesterol, 0.5% cholic (Sinodiets, Siping, China). Hyperlipidemia was characterized by elevated serum levels of TG, TC, and LDL. After developing the hyperlipidemia model in hamsters. Those hamsters were divided into four groups (n=12/group) and were administered with 0.5% CMC-Na, hesperetin (100 mg/kg/day), pectolinarigenin (100 mg/kg/day) or atorvastatin (Avt, 1.0 mg/kg/day), once every 3 days for 7 weeks. All hamsters were also fed HFD for 7 weeks. The doses of hesperetin and pectolinarigenin used in this study were according to previous reports [11]. The dose of Avt used in this study was based on the clinical dosage with a conversion formula from human to hamsters [12]. The body weight and food intake of the hamsters were measured every three or four days. On day 28, blood was collected and the serum biochemical parameters were measured. At the end of the experimental period, the hamsters were fasted overnight and killed. Blood and

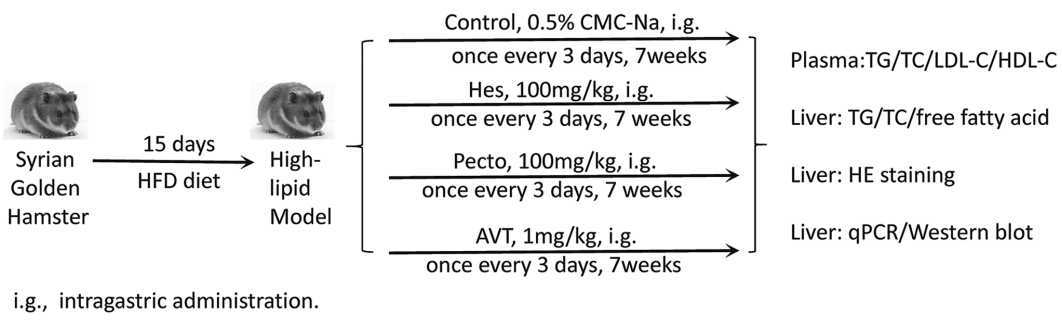

Fig. 1. The whole experimental scheme of animal study. Hes, hesperetin (CAS:520-33-2); Pecto, pectolinarigenin (CAS:520-12-7); Avt, atorvastatin (CAS:134523-03-8); i.g., intragastric administration. Hamsters were fed high-fat diet (HFD) for 15 days plus another 7 weeks during the whole experiment.

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liver samples were collected for biochemical parameter measurement. Body fat such as perirenal fat and epididymal fat were dissected and measured by sensitive balance. Also, partial livers were quickly extracted and frozen in liquid nitrogen and stored at −80°C for western blot assay or quickly fixed in a 10% neutral buffered formalin solution for histopathologic use.

Biochemical analysis

Blood samples were collected and centrifuged at 1,000 g for 10 min at 4°C. The levels of serum TC, TG, LDL, and HDL were determined using specific enzyme-based kits (Jiancheng Biotechnological Institute, Nanjing, China) according to the manufacturer's instructions. All analyses employed an automated analyzer (7020 Clinical Analyzer; Hitachi, Tokyo, Japan). The hepatic lipid including hepatic TC, TG, and free fatty acids were also measured by the same kits. Briefly, the liver samples (~0.1 g) were homogenized by adding phosphate buffer saline (0.01M, pH=7.4), and the supernatants were obtained by centrifuging. Then, the supernatants were further determined according to the manufacturer's instructions to get the TC, TG, and free fatty acid results. The liver index was calculated by the following formula: liver index = (wet weight of liver/body weight) × 100% [13].

Histological analysis

For hematoxylin and eosin (H&E) staining, tissue samples of the liver were dissected and fixed in a 10% neutral buffered formalin fixative, dehydrated in serial alcohol solutions, and embedded in paraffin. All tissues were cut to a thickness of 4 μm, and stained with H&E. All slides were well viewed with an optical microscope (Olympus Optical, Tokyo, Japan), and photographed at a final magnification of ×100.

Quantitative Real-Time PCR (qPCR) analysis

Liver tissue from each group was ground by adding a small amount of liquid nitrogen and the total RNAs were prepared from the liver by using ultra-pure TRIzol reagent according to the manufacturer's instructions (Thermo Fisher, Carlsbad, CA, USA). Reverse transcription was performed on equal amounts of total RNA by using random hexanucleotide primers to produce a cDNA library for each sample. qPCR was run in the ABI ViiA™7 RealTime System (Thermo Fisher) by using SYBR Green Master Mix (Transgen, Beijing, China). Each sample was run in triplicate, and the comparative threshold cycle (Ct) method was used to quantify fold increase ($2^{-\Delta\Delta}$Ct) compared with lean controls. The primer sequences used for qPCR are provided in Table 1.

Western blot analysis

Total liver protein was obtained from hamsters in each group using RIPA Tissue/ Cell Lysis (Solarbio, Beijing, China). The protein content was determined with Bradford Protein Quantitation Assay (Thermo Fisher, Shanghai, China). All primary antibodies were made by a commercial company (QualityYard Biotechnology, Beijing, China). Proteins loading in the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels were obtained from tissue extracts. A total of 20 μg of proteins were separated on 8–12% SDS-PAGE gel. PVDF membrane (Millipore Corp., Bedford, MA, USA) was used for transfer and then blocked for 1 h at room temperature with 5% bovine serum albumin in Tris-buffered saline containing 0.05% Tween 20 (TBST). Subsequently, blots were washed and incubated overnight at 4°C in TBST containing 1% bovine serum albumin with a 1:1,000 dilution of Fasn, Cpt1a, Slc27a1, and Hmgcr antibodies, and 1:5,000 of beta-tubulin antibody (Boster Biological Technology, Co., Ltd., Wuhan, China). Membranes were washed thrice with TBST, incubated with a secondary antibody (1: 10,000 dilutions in TBST containing 1% bovine serum albumin) (Boster Biotech, Wuhan, China) for 60 min at room temperature, and then washed thrice with TBST. After the chemiluminescence reaction (Pierce, Rockford, IL, USA), bands

Table 1. Primers used in this study

	Forward (5'->3')	Forward (5'->3')	Product length	Template
Fasn	GAATCCGCACAGGCTACCAA	AGACCATGGCCAGGTTGAAG	168 bp	XM_005069786.4
Acc1	ATGCTTCTGGGACATCGCAA	CGCCTCCAAAAAGAACTAGCC	139 bp	XM_013118312.3
Scd1	ATCCGCCCTGAAATGAAAGAA	GACATAGTACATAATTCCGAAGAGC	192 bp	NM_001281585.1
Srebp1c	GCACTTTTTGACACGTTTCTTC	CTGTACAGGCTCTCCTGTGG	165 bp	XM_005067682.4
Cpt1a	GACCCCACGAAAACGATGGA	GTACAGGTGCTGGTGCTTCT	72 bp	XM_040750544.1
Srebp2	GAGAGCTGTGAATTTTCCAGTG	CTACAGATGATATCCGGACCAA	137 bp	XM_040752727.1
Hmgcr	GACGGTGACACTTACCATCTGT	GATGCACCGTGTTATGGTGA	145 bp	NM_001281702.1
Hmgcs1	TTTGATGCAGCTGTTTGAGG	CCACCTGTAGGTCTGGCATT	198 bp	XM_013122579.3
Sqs	ATCCCCCACGTCATCACCTA	CGATGGCCATTACCTGTGGA	85 bp	XM_005075273.4
Ldlr	TTGGGTTGATTCCAAACTCC	GATTGGCACTGAAAATGGCT	170 bp	XM_005078537.4
Pparα	CCTGTCTGTTGGGATGTCAC	AGGTAGGCCTCGTGGATTCT	162 bp	XM_040752671.1

were detected by exposing blots to X-ray films for the appropriate period. For quantitative analysis, bands were detected and evaluated for their densities by ImageJ software (National Institute of Mental Health, Bethesda, MD, USA), normalized for beta-tubulin density.

Statistical analysis

Statistical analysis was performed using GraphPad Prism software, version 8.0. Data were expressed as mean ± standard error of mean (SEM). Unpaired *t*-tests were performed for comparison between the two groups. One-way analysis of variance (ANOVA) followed by the Bonferroni procedure was used to compare more than two groups. Differences were considered significant at $P<0.05$.

Results

Establishment of high lipid model using HFD in Golden Syrian hamsters

To establish a high lipid model in hamsters, animals were fed with HFD for 15 days, the body weights were measured and blood was collected from the orbital sinus for biochemical parameter measurement. As can be seen from Fig. 2a, after 15 days of feeding, the body weights showed a significant elevation (161.8 ± 10.1 g vs. 143.0 ± 9.2 g, $P<0.05$). The serum levels of TG, TC, LDL and HDL were 7.57-fold, 2.87-fold, 12.40-fold and 1.18-fold higher than those in the "before feeding" group, respectively (After: 13.25 ± 3.82 mmol/L vs. Before: 1.75 ± 0.24 mmol/L; After: 19.56 ± 3.66 mmol/L vs. Before: 6.81 ± 0.74 mmol/L; After: 8.81 ± 0.83 mmol/L vs. Before: 0.71 ± 0.09 mmol/L; After: 2.75 ± 0.42 mmol/L vs. Before: 2.32 ± 0.15 mmol/L, $P<0.05$). These data demonstrate that we have established a well-high lipid model in Golden Syrian hamsters. We don't use negative control instead of using self-control when feeding with HFD because there were several reports claiming that more than 1.3-fold changes in serum TC, TG, and LDL are enough to establish a well-high lipid model [14, 15].

Effect of hesperetin and pectolinarigenin on serum and hepatic lipid contents

The effect of hesperetin and pectolinarigenin on body weight and adiposity index in hamsters fed with HFD were evaluated. The mean body weight was significantly decreased in the pectolinarigenin group compared to the control group after 35 days, whereas hesperetin and atorvastatin (Avt) did not reduce the increased body weight through the whole experimental process (Fig. 3a). This result indicated that only pectolinarigenin affects the body weight gain of hamsters. However, food intake results showed no significant changes in food intake per day (g) among all experimental groups (Figs. 3b and c). The adiposity index was calculated according to the following formula: 100 × (epididymal fat weight + perirenal fat weight)/body weight. The adiposity index showed significant decreases in pectolinarigenin and Avt groups compared to that in the control and hesperetin groups (Pecto: 2.81 ± 0.30 vs. Control: 3.49 ± 0.37 g,

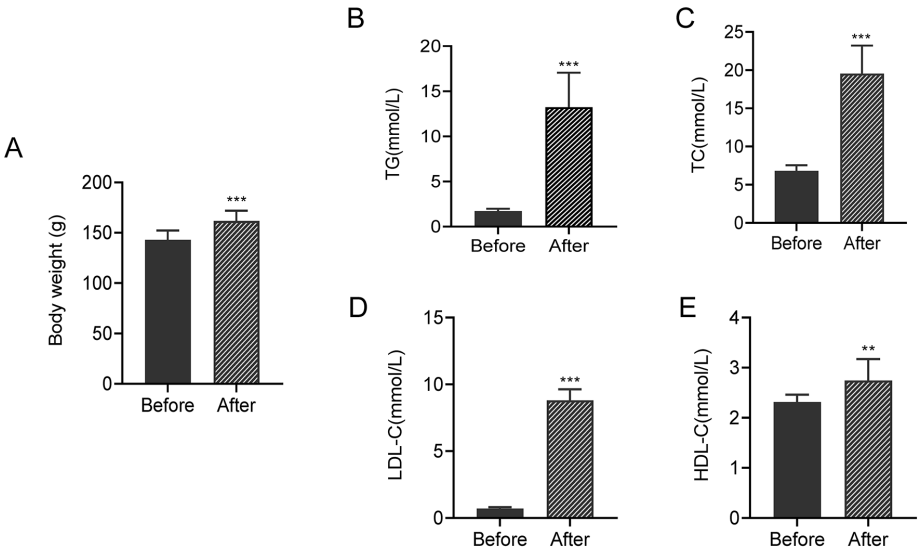

Fig. 2. High-fat diet-induced hyperlipidemia and hepatic steatosis in Golden Syrian hamsters. (a) Effect of the high-fat diet on the change in body weight. (b–c) Effect of the high-fat diet on the changes in serum triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL), and high-density lipoprotein cholesterol (HDL). Data are mean ± SD (n=48). Before, before feeding with high fat diet; After, after feeding with high fat diet for 15 days (*$P<0.05$, **$P<0.01$, ***$P<0.001$).

$P<0.05$; Avt: 3.06 ± 0.48 vs. Control: 3.49 ± 0.37 g, $P<0.001$), indicating that pectolinarigenin and Avt treatments have inhibitory effects on HFD-induced adipogenesis (Fig. 3d).

Next, we investigated if hesperetin or pectolinarigenin can lower serum and hepatic lipid contents in hamsters fed with HFD. Compared to the control group, the mean serum levels of TG and TC were decreased by 50.4 and 11.3% in pectolinarigenin group and 42.5 and 12.7% in Avt group on day 28, respectively. However, hesperetin treatment resulted in elevations in serum TC and LDL on day 28. This phenomenon may be attributed to the negative feedback effect of HFD feeding (Fig. 3e). Compared to the control group, the mean serum levels of TG and TC were decreased by 37.3 and 31.5% in pectolinarigenin group and 26.7 and 58.5% in Avt group on day 49, respectively. Moreover, the mean serum level of LDL was decreased by 38.0% in Avt group compared to that in the control group. However, hesperetin treatment only induced a reduction in the serum TC level (Fig. 3f).

Compared to the control group, the mean levels of the liver index, hepatic TC, TG, and free fatty acid in pectolinarigenin and Avt groups decreased by 29.3 and 18.9%, 11.7 and 52.8%, 25.7 and 23.6%, 30.6 and 34.5%, respectively (Fig. 3g), however, liver index, hepatic TC and free fatty acid levels showed no significant differences in hesperetin group in comparison to the control group, and only the TG level was decreased by hesperetin treatment. These results indicated that pectolinarigenin and Avt both could lower serum and hepatic lipid contents induced by HFD in hamsters, whereas hesperetin had only a minor effect on lowering hepatic lipid contents.

Histopathological evaluations

To further investigate the effect of hesperetin and pectolinarigenin on hepatic changes, H&E staining was applied to evaluate the pathologic injuries and hepatic steatosis. It was found that in the control group, hepatic steatosis appeared, namely, many small lipid vesicles in the liver were seen (Fig. 4a). After treatment with pectolinarigenin and Avt, the degree of hepatic steatosis was improved, evidenced by significantly fewer lipid vesicles (Figs. 4c and d). Nonetheless, the degree of hepatic steatosis had fewer changes in lipid accumulation in the hesperetin group in comparison to the control group, illustrated by H&E (Fig. 4b) staining, suggesting that pectolinarigenin and Avt have protective effects on liver lipid droplet accumulation induced by HFD in hamsters.

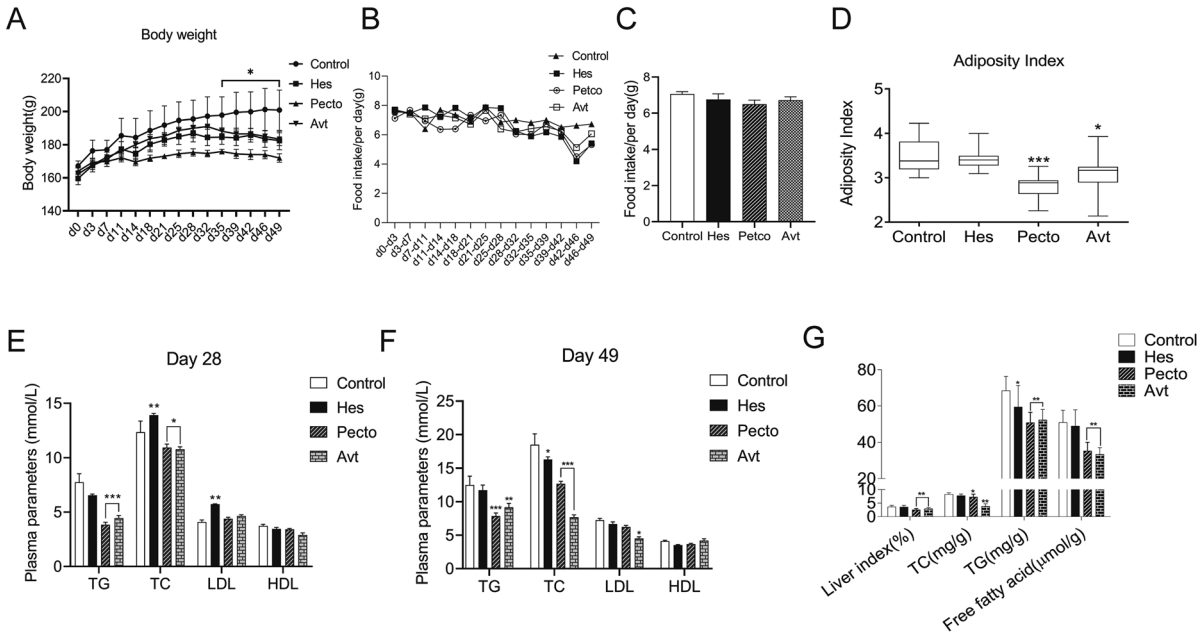

Fig. 3. Effects of hesperetin and pectolinarigenin on body weight and serum lipid contents. (a) Effects of hesperetin and pectolinarigenin on the change in body weight. (b) Effects of hesperetin and pectolinarigenin on the change in food intake. (c) Effects of hesperetin and pectolinarigenin on the change in food intake per day during the whole experimental process. (d) Effects of hesperetin and pectolinarigenin on the change in adiposity index. The adiposity index was calculated according to the following formula: 100 × (epididymal fat weight + perirenal fat weight)/body weight. (e) Effects of hesperetin and pectolinarigenin on the change in serum lipid parameters on day 28. (f) Effects of hesperetin and pectolinarigenin on the change in serum lipid parameters on day 49. (g) Effects of hesperetin and pectolinarigenin on the change in hepatic lipid parameters after the whole experiment (Hes, hesperetin; Pecto, pectolinarigenin; Avt, atorvastatin. *$P<0.05$, **$P<0.01$, ***$P<0.001$).

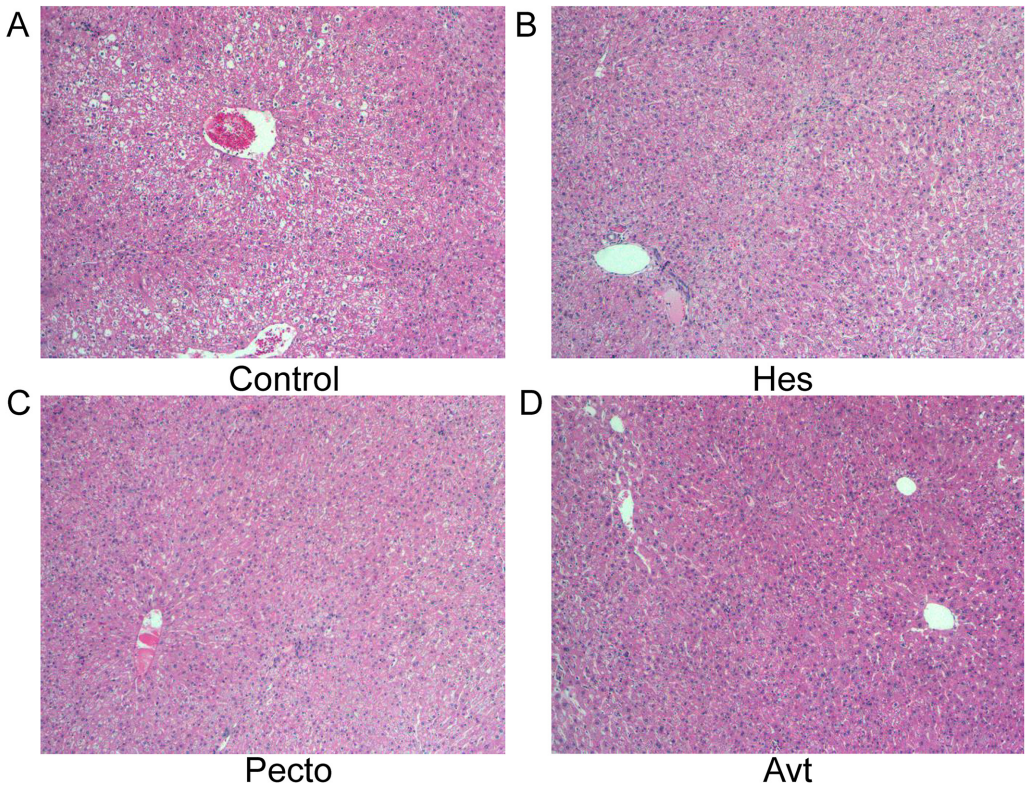

Fig. 4. Effect of hesperetin and pectolinarigenin on the morphology of liver tissues. (a-d) Representative hematoxylin and eosin (H&E) staining of hamsters liver in control, hesperetin (Hes), pectolinarigenin (Pecto), atorvastatin (Avt), respectively (Magnification: 100 ×).

Effects of hesperetin and pectolinarigenin on the hepatic genes involved in lipid metabolism

To investigate the effects of hesperetin and pectolinarigenin on the hepatic genes involved in lipid metabolism, several key genes involved in fatty acid and cholesterol metabolism were checked. For genes involved in fatty acid metabolism (Fig. 5A), treatment with pectolinarigenin and Avt both reduced the mRNA levels of acetyl CoA carboxylase 1 (*Acc1*), *Fasn*, stearoyl-CoA desaturase 1 (*Scd1*), and increased the mRNA levels of *Cpt1a* and peroxisome proliferator-activated receptor α (*Pparα*). However, only Avt significantly reduced the mRNA levels of *Srebp1c*.

For genes involved in cholesterol metabolism (Fig. 5B), hesperetin and pectolinarigenin both showed no significant changes in the mRNA levels of *Srebp2*, 3-hydroxy-3-methylglutaryl-CoA synthase 1 (*Hmgcs1*), *Sqs*, and *Ldlr* compared to those in the control group (P>0.05). However, treatment with hesperetin and pectolinarigenin decreased the mRNA level of *Hmgcr* by ~44% and ~47%, respectively. Interestingly. Avt significantly increased the mRNA levels of *Srebp2*, *Ldlr*, and *Hmgcr* by ~377%, 287%, and 86%, respectively. These data indicated that compared to hesperetin treatment, pectolinarigenin treatment showed stronger in-

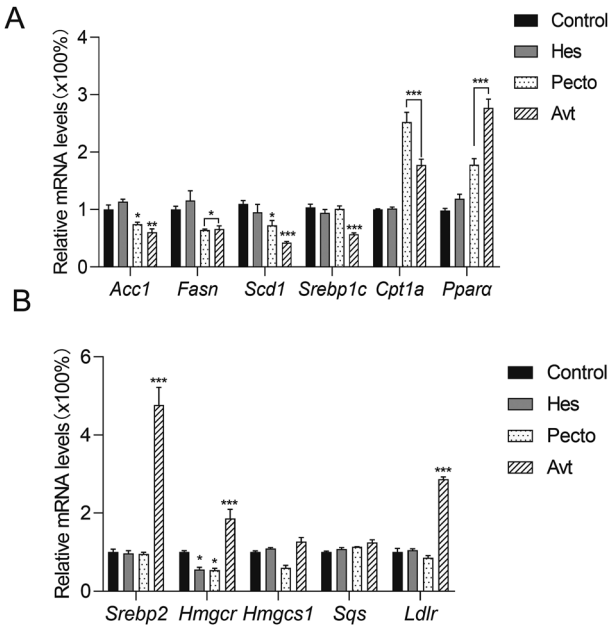

Fig. 5. Effects of hesperetin and pectolinarigenin on the hepatic mRNA levels involved in (A) fatty acid metabolism and (B) cholesterol metabolism (Hes, hesperetin; Pecto, pectolinarigenin; Avt, atorvastatin. *P<0.05, **P<0.01, ***P<0.001).

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hibitory effects on genes involved in fatty acid biosynthesis and promotional effects on genes involved in fatty acid oxidation. However, hesperetin and pectolinarigenin both showed fewer effects on cholesterol metabolism although there was an inhibitory effect of pectolinarigenin on the mRNA expression of *Hmgcr*.

Effects of hesperetin and pectolinarigenin on the hepatic proteins involved in lipid metabolism

To reveal the potential mechanism involved in the effects of pectolinarigenin on hyperlipidemia and hepatic steatosis induced by HFD in hamsters, the protein expressions of Fasn, Cpt1a, Slc27a1, and Hmgcr were investigated. As can be seen in Fig. 6, pectolinarigenin and Avt decreased the protein expression levels of Fasn and Slc27a1 by ~35 and ~34%, ~36 and ~68%, respectively. However, both hesperetin and pectolinarigenin treatments showed no significant changes in hepatic Hmgcr levels (P>0.05), whereas Avt treatment reduced the hepatic Hmgcr expressions (Figs. 6b, d and e). Additionally, it was found that there were marked elevations in levels of Cpt1a in the pectolinarigenin and Avt groups. Unlike Fasn, Slc27a1, and Hmgcr that is responsible for lipid biosynthesis, Cpt1a is a key enzyme responsible for fatty acid oxidation. Pectolinarigenin and Avt increased the protein expression levels of Cpt1a by ~54 and ~49%, respectively (Fig. 6b). These data suggested that pectolinarigenin and Avt treatment ameliorated hyperlipidemia and hepatic steatosis by inhibiting the protein expressions involved in lipid transport and biosynthesis and promoting the protein expressions involved in fatty acid oxidation.

Discussion

The most reported activity of pectolinarigenin is its anti-inflammatory effect [16], it was found that pectolinarigenin was capable of suppressing lipopolysaccharide (LPS)-induced inflammation via NFκB and MAPK pathways in various types of cells [17]. Pectolinarigenin was also found to inhibit cyclooxygenase-2 (COX-2)-mediated prostaglandin E2 (PGE2) and 5-lipoxygenase (5-LOX)-mediated leukotriene (LT) production in LPS-treated RAW 264.7 cells and A23187-treated rat basophilic leukemia (RBL-1) cells, respectively. However, pectolinarigenin and its structural analogs such as hesperetin and hispidulin have also exhibited lipid-lowering effects *in vitro* and *in vivo* [4, 18]. Thus, we compared the lipid-lowering effects of hesperetin and pectolinarigenin in this study.

Although HFD is efficient in inducing hyperlipidemia in rodent models, there are some different characteristics of hyperlipidemia between mice and human, for example, LDL represents the major fraction of plasma cholesterol in human, whereas HDL represents the major fraction of plasma cholesterol in mice [19]. Sometimes, rodents such as mice and rats are not easy to develop high-lipid animal models. Unlike rats and mice, Golden Syrian hamsters have a large proportion of the circulating lipoproteins being the non-HDL form, they possess cholesteryl ester transport protein (CETP), receptor-mediated uptake of LDL lipoproteins via the LDL receptor. Accordingly, hamsters quickly develop hypercholesterolemia and hypertriglyceridemia when fed a

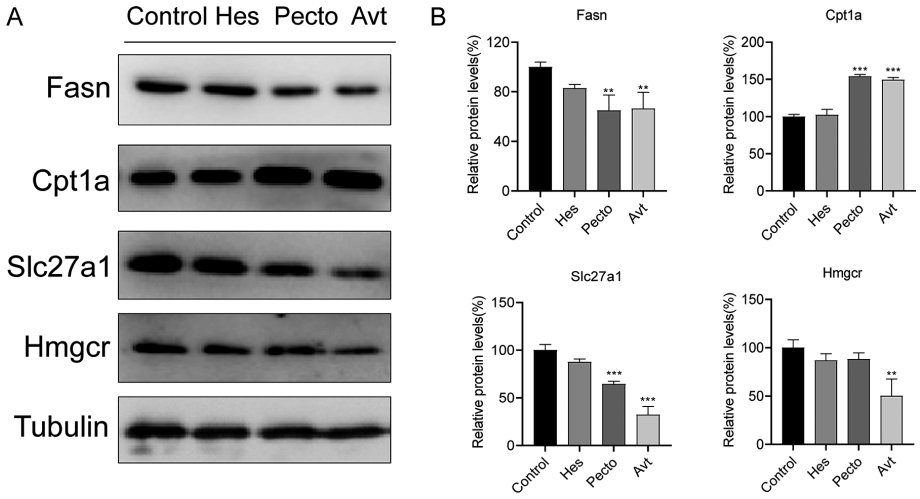

Fig. 6. Effects of hesperetin and pectolinarigenin on the protein levels of fatty-acid synthase (Fasn), carnitine palmitoyltransferase 1A (Cpt1a), solute carrier family 27 member 1 (Slc27a1), and Hmgcr. (a) Protein expressions of Fasn, Cpt1a, Slc27a1, and Hmgcr in hamsters liver. (b–e) Relative expression ratio of Fasn, Cpt1a, Slc27a1, and Hmgcr, respectively. Bands were detected and evaluated for their densities by Image J software, normalized for b-tubulin densities (Hes, hesperetin; Pecto, pectolinarigenin; Avt, atorvastatin. *P<0.05, **P<0.01, ***P<0.001).

cholesterol-rich diet. In addition, hamsters are obesity-prone and quickly develop insulin resistance when fed a high-fat high carbohydrate diet. These characteristics indicate that the hamsters are an excellent species for the assessment of drugs with combined efficacy on hypertriglyceridemia and hypercholesterolemia [8]. Thus, in this study, we developed a HFD-induced hyperlipidemia model in hamsters. Apart from hyperlipidemia, liver steatosis was also seen in the model group. This result can be explained that HFD-induced hepatic lipid accumulation and increased hepatic lipid levels [20].

As a statins' family member, Avt is widely used as a lipid-lowering medication in clinical. However, statins such as Avt also cause side effects, including muscle/joint pain and increased risk of diabetes [21]. In our study, unlike hesperetin, pectolinarigenin was capable of decreasing the TC, TG, and free fatty acid levels in serum and liver tissue, as well as the liver index. Besides, it was found that pectolinarigenin treatment decreased hepatic lipid levels to a greater extent than hesperetin treatment in the liver. Interestingly, Avt treatment also showed a decreased hepatic lipid level compared to that in the control group, this result was consistent with the previous report [22], which can be explained that Avt inhibits the neutrophils chemotaxis and decreases the pro-inflammatory cytokines induced by HFD, thereby ameliorating the hepatic steatosis process. Besides, when comparing the serum lipid profiles among hesperetin, pectolinarigenin, and Avt groups, it was found that both hesperetin and pectolinarigenin treatments showed no changes in the serum levels of LDL and HDL. The mechanism of Avt for lowering serum LDL was reported that Avt inhibits cholesterol synthesis and subsequently stimulates sterol regulatory element-binding protein-2 (SREBP2) transcription activity, upregulates LDLR, and increases LDL uptake from serum. Thus, it seems that hesperetin and pectolinarigenin were not capable of stimulating Srebp2 or upregulating Ldlr. Our qPCR results also showed that hesperetin and pectolinarigenin both showed no effects on the mRNA expressions of *Srebp2* and *Ldlr*. On the other hand, although the mRNA level of *Hmgcr* was downregulated by pectolinarigenin, the protein level of Hmgcr was not changed, and the serum TC lowering effect of pectolinarigenin may attribute to other unknown mechanisms. Nonetheless, compared to hesperetin treatment, pectolinarigenin treatment inhibited the hepatic mRNA expressions of *Acc1*, *Fasn*, and *Scd1*, and increased the hepatic mRNA expressions of *Cpt1a* and *Pparα*. These results suggested that the TG-lowering effect of pectolinarigenin may attribute to the inhibitory effects on genes involved in fatty acid biosynthesis and the promotional impact on genes involved in fatty acid oxidation.

It was reported that some flavonoid compounds, such as hispidulin and luteolin, which both exhibit similar structures to pectolinarigenin, targeted peroxisome proliferators-activated receptor γ (PPARγ) and induced PPARγ activation [23, 24]. PPARγ is a key transcription factor involved in lipid metabolism, PPARγ activation induces decreased lipid biosynthesis and increased lipid metabolism [25]. Additionally, it was found that PPARγ activation also repressed fatty acid import and lipid synthesis through inhibition of SLC27A1 expression [26]. Thus, it's reasonable to speculate that pectolinarigenin may also target PPARγ and regulate protein expressions associated with lipid transport, biosynthesis, and metabolism.

Fasn is a key protein responsible for catalyzing the synthesis of fatty acid, that is, catalyzing the synthesis of palmitate from acetyl-CoA and malonyl-CoA, in the presence of nicotinamide adenine dinucleotide phosphate hydrogen (NADPH), into long-chain saturated fatty acids [27]. Cpt1a is the key enzyme responsible for fatty acid oxidation and its deficiency results in a decreased rate of fatty acid beta-oxidation [28]. Slc27a1 is a key protein responsible for long-chain fatty acid transport and Hmgcr is the rate-limiting enzyme for cholesterol synthesis [29, 30]. We next investigated the effect of hesperetin and pectolinarigenin on the protein expressions of Fasn, Cpt1a, Slc27a1, and Hmgcr. It was found that only pectolinarigenin treatment inhibited the expressions of Fasn and Slc27a1, improved the expression of Cpt1a, and subsequently caused the downregulations of fatty acid synthesis and upregulations of fatty acid oxidation, which further explained the negative emergence of hyperlipidemia and hepatic steatosis in pectolinarigenin group. However, hesperetin and pectolinarigenin both have no significant effects on the expression of hepatic Hmgcr, which might be attributed to the fact that hesperetin and pectolinarigenin both show minor effects on SREBP-2's expression.

In summary, we compared the protective effects of hesperetin and pectolinarigenin on HFD-induced hyperlipidemia and hepatic steatosis in Golden Syrian hamsters, this study demonstrated that pectolinarigenin was effective in protecting against hyperlipidemia and hepatic steatosis induced by HFD. The mechanism might be due to the suppression of protein expressions involved in lipid transport and biosynthesis and improvement of protein expressions involved in lipid metabolism, thereby resulting in a relatively normal lipid level in serum and liver.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

References

1. Chao HW, Chao SW, Lin H, Ku HC, Cheng CF. Homeostasis of glucose and lipid in non-alcoholic fatty liver disease. Int J Mol Sci. 2019; 20: 298. [Medline] [CrossRef]
2. Lee HJ, Venkatarame Gowda Saralamma V, Kim SM, Ha SE, Raha S, Lee WS, et al. Pectolinarigenin induced cell cycle arrest, autophagy, and apoptosis in gastric cancer cell via pi3k/akt/mtor signaling pathway. Nutrients. 2018; 10: 1043. [Medline] [CrossRef]
3. Kuang W, Zhang X, Lan Z. Flavonoids extracted from Linaria vulgaris protect against hyperlipidemia and hepatic steatosis induced by western-type diet in mice. Arch Pharm Res. 2018; 41: 1190–1198. [Medline] [CrossRef]
4. Zhang Y, Wan C, Song Z, Meng W, Wang S, Lan Z. Pectolinarigenin reduces the expression of sterol regulatory element-binding proteins and cellular lipid levels. Biosci Biotechnol Biochem. 2022; 86: 1220–1230. [Medline] [CrossRef]
5. Kumkarnjana S, Suttisri R, Nimmannit U, Koobkokkruad T, Pattamadilok C, Vardhanabhuti N. Anti-adipogenic effect of flavonoids from Chromolaena odorata leaves in 3T3-L1 adipocytes. J Integr Med. 2018; 16: 427–434. [Medline] [CrossRef]
6. Wilcox LJ, Borradaile NM, de Dreu LE, Huff MW. Secretion of hepatocyte apoB is inhibited by the flavonoids, naringenin and hesperetin, via reduced activity and expression of ACAT2 and MTP. J Lipid Res. 2001; 42: 725–734. [Medline] [CrossRef]
7. Kim HK, Jeong TS, Lee MK, Park YB, Choi MS. Lipid-lowering efficacy of hesperetin metabolites in high-cholesterol fed rats. Clin Chim Acta. 2003; 327: 129–137. [Medline] [CrossRef]
8. Fazio S, Linton MF. Mouse models of hyperlipidemia and atherosclerosis. Front Biosci. 2001; 6: D515–D525. [Medline] [CrossRef]
9. Dalbøge LS, Pedersen PJ, Hansen G, Fabricius K, Hansen HB, Jelsing J, et al. A hamster model of diet-induced obesity for preclinical evaluation of anti-obesity, anti-diabetic and lipid modulating agents. PLoS One. 2015; 10: e0135634. [Medline] [CrossRef]
10. Choi WH, Gwon SY, Ahn J, Jung CH, Ha TY. Cooked rice prevents hyperlipidemia in hamsters fed a high-fat/cholesterol diet by the regulation of the expression of hepatic genes involved in lipid metabolism. Nutr Res. 2013; 33: 572–579. [Medline] [CrossRef]
11. Kim HJ, Jeon SM, Lee MK, Cho YY, Kwon EY, Lee JH, et al. Comparison of hesperetin and its metabolites for cholesterol-lowering and antioxidative efficacy in hypercholesterolemic hamsters. J Med Food. 2010; 13: 808–814. [Medline] [CrossRef]
12. Nair AB, Jacob S. A simple practice guide for dose conversion between animals and human. J Basic Clin Pharm. 2016; 7: 27–31. [Medline] [CrossRef]
13. Zou Y, Li J, Lu C, Wang J, Ge J, Huang Y, et al. High-fat emulsion-induced rat model of nonalcoholic steatohepatitis. Life Sci. 2006; 79: 1100–1107. [Medline] [CrossRef]
14. Li J, Wu H, Liu Y, Yang L. High fat diet induced obesity model using four strainsof mice: Kunming, C57BL/6, BALB/c and ICR. Exp Anim. 2020; 69: 326–335. [Medline] [CrossRef]
15. Naseri E, Xiangyu K, Hu C, Ayaz A, Rahmani MM, Nasim M, et al. Bok-choy promotes growth performance, lipid metabolism and related gene expression in Syrian golden hamsters fed with a high-fat diet. Food Funct. 2020; 11: 2693–2703. [Medline] [CrossRef]
16. Cheriet T, Ben-Bachir B, Thamri O, Seghiri R, Mancini I. Isolation and Biological Properties of the Natural Flavonoids Pectolinarin and Pectolinarigenin-A Review. Antibiotics (Basel). 2020; 9: 417. [Medline] [CrossRef]
17. Heimfarth L, Nascimento LDS, Amazonas da Silva MJ, Lucca Junior W, Lima ES, Quintans-Junior LJ, et al. Neuroprotective and anti-inflammatory effect of pectolinarigenin, a flavonoid from Amazonian Aegiphila integrifolia (Jacq.), against lipopolysaccharide-induced inflammation in astrocytes via NFκB and MAPK pathways. Food Chem Toxicol. 2021; 157: 112538. [Medline] [CrossRef]
18. Wu X, Xu J. New Role of Hispidulin in Lipid Metabolism: PPARα Activator. Lipids. 2016; 51: 1249–1257. [Medline] [CrossRef]
19. Minniti ME, Pedrelli M, Vedin LL, Delbès AS, Denis RGP, Öörni K, et al. Insights from liver-humanized mice on cholesterol lipoprotein metabolism and lxr-agonist pharmacodynamics in humans. Hepatology. 2020; 72: 656–670. [Medline] [CrossRef]
20. Gauthier MS, Favier R, Lavoie JM. Time course of the development of non-alcoholic hepatic steatosis in response to high-fat diet-induced obesity in rats. Br J Nutr. 2006; 95: 273–281. [Medline] [CrossRef]
21. Selva-O'Callaghan A, Alvarado-Cardenas M, Pinal-Fernández I, Trallero-Araguás E, Milisenda JC, Martínez MA, et al. Statin-induced myalgia and myositis: an update on pathogenesis and clinical recommendations. Expert Rev Clin Immunol. 2018; 14: 215–224. [Medline] [CrossRef]
22. Martín-Castillo A, Castells MT, Adánez G, Polo MT, Pérez BG, Ayala I. Effect of atorvastatin and diet on non-alcoholic fatty liver disease activity score in hyperlipidemic chickens. Biomed Pharmacother. 2010; 64: 275–281. [Medline] [CrossRef]
23. Ding L, Jin D, Chen X. Luteolin enhances insulin sensitivity via activation of PPARγ transcriptional activity in adipocytes. J Nutr Biochem. 2010; 21: 941–947. [Medline] [CrossRef]
24. Han M, Gao H, Ju P, Gao MQ, Yuan YP, Chen XH, et al. Hispidulin inhibits hepatocellular carcinoma growth and metastasis through AMPK and ERK signaling mediated activation of PPARγ. Biomed Pharmacother. 2018; 103: 272–283. [Medline] [CrossRef]
25. Tian L, Wang C, Hagen FK, Gormley M, Addya S, Soccio R, et al. Acetylation-defective mutant of Pparγ is associated with decreased lipid synthesis in breast cancer cells. Oncotarget. 2014; 5: 7303–7315. [Medline] [CrossRef]
26. Shi K, Li R, Xu Z, Zhang Q. Identification of crucial genetic factors, such as ppargamma, that regulate the pathogenesis of fatty liver disease in dairy cows is imperative for the sustainable development of dairy industry. Animals (Basel). 2020; 10: 639. [Medline]
27. Menendez JA, Lupu R. Fatty acid synthase (FASN) as a therapeutic target in breast cancer. Expert Opin Ther Targets. 2017; 21: 1001–1016. [Medline] [CrossRef]
28. Hao F, Tian M, Zhang X, Jin X, Jiang Y, Sun X, et al. Butyrate enhances CPT1A activity to promote fatty acid oxidation and iTreg differentiation. Proc Natl Acad Sci USA. 2021; 118: e2014681118. [Medline] [CrossRef]
29. Gimeno RE. Fatty acid transport proteins. Curr Opin Lipidol. 2007; 18: 271–276. [Medline] [CrossRef]
30. Cánovas A, Quintanilla R, Gallardo D, Díaz I, Noguera JL, Ramírez O, et al. Functional and association studies on the pig HMGCR gene, a cholesterol-synthesis limiting enzyme. Animal. 2010; 4: 224–233. [Medline] [CrossRef]