Effects of Alfalfa Saponins on mRNA Expression of Low-Density Lipoprotein Receptor and ATP-Binding Cassette Transporters in Rat Liver and Hepatocytes (Postprint)

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Abstract

This study aimed to investigate the effects of alfalfa saponins on the mRNA expression levels of key genes in the cholesterol clearance and transport pathways, including low-density lipoprotein receptor (LDLR), ATP-binding cassette transporter G5 (ABCG5), and ATP-binding cassette transporter G8 (ABCG8), in rat liver and rat liver cells (BRL cells), and to preliminarily explore the molecular mechanism by which alfalfa saponins regulate cholesterol clearance and transport at both individual and cellular levels. A high-fat diet was used to establish a rat hyperlipidemia model to determine the effects of alfalfa saponins on serum indices [total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) levels] and hepatic mRNA expression levels of LDLR, ABCG5, and ABCG8 in normal and hyperlipidemic rats; high-glucose DMEM medium was used to establish a BRL cell steatosis model to determine the effects of alfalfa saponin concentrations on BRL cell viability and the effects of alfalfa saponins on mRNA expression levels of LDLR, ABCG5, and ABCG8 in normal and steatotic cells. The results showed that: 1) alfalfa saponins significantly decreased the serum levels of TG, TC, and LDL-C in hyperlipidemic rats ($P<0.05$); 2) alfalfa saponins significantly upregulated the hepatic mRNA expression levels of LDLR, ABCG5, and ABCG8 in normal rats and ABCG5 and ABCG8 in hyperlipidemic rats ($P<0.05$); 3) supplementation with 200 and 250 g/mL alfalfa saponins significantly increased BRL cell viability ($P<0.05$); and 4) alfalfa saponins significantly upregulated the mRNA expression levels of LDLR, ABCG5, and ABCG8 in normal BRL cells ($P<0.05$), but had no significant effect on the mRNA expression levels of these genes in steatotic BRL cells ($P>0.05$). These results suggest that alfalfa saponins can increase cholesterol clearance and transport within hepa-

tocytes by regulating the expression of LDLR, ABCG5, and ABCG8 mRNA, thereby reducing systemic cholesterol levels.

Full Text

Effects of Alfalfa Saponins on mRNA Expressions of Low-Density Lipoprotein Receptor and Adenosine Triphosphate Binding Cassette Transporters in Liver and Liver Cells of Rats

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Abstract: This experiment was conducted to investigate the effects of alfalfa saponins on mRNA expressions of low-density lipoprotein receptor (LDLR), adenosine triphosphate binding cassette transporter G5 (ABCG5), and adenosine triphosphate binding cassette transporter G8 (ABCG8) in rat liver and rat liver cells (BRL cells), and to explore the molecular mechanism by which alfalfa saponins regulate cholesterol clearance and transport at both individual and cellular levels. A hyperlipidemic rat model was established using a high-fat diet to determine the effects of alfalfa saponins on serum indices [total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) contents] and hepatic mRNA expressions of LDLR, ABCG5, and ABCG8 in normal and hyperlipidemic rats. Additionally, a hyperlipidemic cell model was established using high-glucose DMEM culture medium to determine the effects of alfalfa saponin concentration on BRL cell viability and the effects of alfalfa saponins on mRNA expressions of LDLR, ABCG5, and ABCG8 in normal and hyperlipidemic cells. The results showed that: (1) alfalfa saponins significantly decreased serum TG, TC, and LDL-C contents in hyperlipidemic rats ($P<0.05$); (2) alfalfa saponins significantly upregulated hepatic mRNA expressions of LDLR, ABCG5, and ABCG8 in normal rats and those of ABCG5 and ABCG8 in hyperlipidemic rats ($P<0.05$); (3) supplementation with 200 and 250 g/mL alfalfa saponins significantly increased BRL cell viability ($P<0.05$); and (4) alfalfa saponins significantly upregulated mRNA expressions of LDLR, ABCG5, and ABCG8 in normal BRL cells ($P<0.05$), but had no significant effects on those in hyperlipidemic BRL cells ($P>0.05$). These results suggest that alfalfa saponins may enhance intracellular cholesterol clearance and transport by regulating mRNA expressions of LDLR, ABCG5, and ABCG8, thereby reducing cholesterol content in the body.

Key words: alfalfa saponins; rat; BRL cell; low-density lipoprotein receptor; adenosine triphosphate binding cassette transporter G5; adenosine triphosphate binding cassette transporter G8; mRNA expression

With the development of social modernization, people' s quality of life has continuously improved, but this has also brought adverse health effects. Dyslipidemia, particularly elevated dietary cholesterol, is a major factor contributing to atherosclerosis and coronary heart disease. Statistics indicate that approximately 10 million people worldwide die from hyperlipidemia each year. In China, about 90 million people suffer from hyperlipidemia, and this population continues to grow with rising living standards, showing a trend toward younger age groups. Numerous large-scale clinical trials have confirmed that cholesterol reduction can significantly improve myocardial ischemia symptoms, relieve angina, and improve cardiac function in cardiovascular disease patients, thereby reducing the incidence of cardiovascular diseases.

Traditional Chinese medicine and plant extracts have a long history of application in lipid-lowering therapy, offering abundant resources, significant effects, minimal side effects, and good long-term tolerance. Plant extracts have become a major focus in lipid-lowering drug research and development due to their wide availability, low toxicity, and notable lipid-lowering effects. Many studies have shown that active compounds in plants, such as saponins, sterols, flavonoids, alkaloids, and terpenoids, can reduce cholesterol levels in the body, with saponins showing the most pronounced lipid-lowering effects and attracting the most attention. Alfalfa saponins are biologically active compounds extracted from alfalfa (*Medicago sativa*), consisting of cyclic acetal compounds formed by the dehydration condensation of hydroxyl groups in sugars or non-sugar compounds, with a structure of pentacyclic triterpenoid compounds. Numerous studies have demonstrated that alfalfa saponins can reduce cholesterol content in animals and exhibit good anti-atherosclerotic effects, making them ideal raw materials for cholesterol-lowering drugs and green feed additives.

The body maintains cholesterol homeostasis primarily through cholesterol synthesis, intestinal absorption, and excretion via bile and feces. The adenosine triphosphate binding cassette (ABC) transporter family mediates the unidirectional transmembrane transport of cholesterol to the extracellular space. Additionally, the low-density lipoprotein receptor pathway plays a crucial role in reducing serum cholesterol content, regulating low-density lipoprotein metabolism, and maintaining normal blood cholesterol levels. This study aimed to investigate the effects of alfalfa saponins on mRNA expressions of low-density lipoprotein receptor (LDLR), adenosine triphosphate binding cassette transporter G5 (ABCG5), and adenosine triphosphate binding cassette transporter G8 (ABCG8) in normal and hyperlipidemic rats and normal and hyperlipidemic rat liver cells (BRL cells), and to explore the molecular mechanism by which alfalfa saponins regulate cholesterol clearance and transport, thereby reducing cholesterol levels. This research provides a theoretical basis for the extensive application of alfalfa saponins in the production of safe, green, and healthy animal products.

1.1.1 Experimental Design

Thirty-two male healthy specific-pathogen-free (SPF) Sprague-Dawley rats weighing (191.41±16.01) g were purchased from the Henan Experimental Animal Center. After one week of acclimation feeding, all rats were fasted overnight, weighed, and blood was collected from the tail artery to measure serum total cholesterol (TC) content. Based on serum TC content and body weight, the rats were randomly divided into two groups: a normal group and a hyperlipidemic group, with 16 rats in each group. There were no statistically significant differences in serum TC content and body weight between the two groups ($P>0.05$). A hyperlipidemic rat model was established by feeding a high-fat diet. The normal group was fed a basal diet, while the hyperlipidemic group was fed a high-fat diet. The high-fat diet composition was: 1.0% cholesterol, 0.1% pig bile salts, 10.0% lard, 5.0% egg yolk powder, 5.0% whole milk powder, and 78.9% basal diet. The modeling period lasted four weeks, during which body weight was measured weekly and blood was collected from the tail artery at the end of week 4. After successful modeling, both the normal and hyperlipidemic groups were further randomly divided into two subgroups of eight rats each based on serum TC content and body weight. The experimental design was as follows: normal control group (basal diet, 2 mL distilled water gavage daily at 09:00), normal saponin group (basal diet, 240 mg/kg alfalfa saponins in 2 mL gavage daily at 09:00), hyperlipidemic model group (high-fat diet, 2 mL distilled water gavage daily at 09:00), and hyperlipidemic saponin group (high-fat diet, 240 mg/kg alfalfa saponins in 2 mL gavage daily at 09:00). The experimental period lasted seven days. All rats had free access to water and feed, with all feed and drinking water sterilized. Alfalfa saponins were provided by Baon Biological Technology Co., Ltd. in Cangzhou, Hebei Province, with a total saponin content of 51%.

1.1.2 Sample Collection and Preparation

At the end of the experiment, all rats were fasted overnight, anesthetized with ether, and blood was collected from the tail. Blood samples were stored at 4 °C overnight, then centrifuged at 3,000 r/min for 10 min to separate serum, which was aliquoted into EP tubes and stored at -20 °C for later analysis. The abdominal cavity was opened along the midline, the liver was removed, blotted dry, and weighed. Each liver was divided into two halves: one half was wrapped in aluminum foil and stored at -20 °C, while the other half was immediately frozen in liquid nitrogen and stored at -80 °C.

1.1.3 Serum Index Determination

Serum TC, triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) contents were measured using an automatic biochemical analyzer according to the kit instructions.

1.2 Cell Experiments

BRL cells were selected as the experimental material and purchased from the Cell Resource Center of the Shanghai Institute of Life Sciences, Chinese Academy of Sciences.

1.2.1 Cell Counting

Cell counting was performed as follows: (1) Prepare a cell suspension of appropriate concentration; (2) Clean the counting chamber and cover slip, and place the cover slip on the counting chamber; (3) Aspirate 15 L of cell suspension and slowly add it along the edge of the cover slip until the suspension fills the space between the cover slip and counting chamber. Allow to stand for approximately 3 min, ensuring no bubbles form under the cover slip and that the suspension does not flow into adjacent grooves; (4) Count all cells in the four large squares of the counting chamber, counting only cells on the left and upper borders to avoid double-counting. Count cell clusters as single cells under the microscope. If cell clusters exceed 10%, the suspension should be re-prepared; (5) Cell count (cells/mL) = (total cells in four large squares/4) × 10 .

1.2.2 BRL Cell Culture

BRL cells were inoculated into 25 cm^2 culture flasks with high-glucose DMEM medium containing 10% fetal bovine serum, just enough to cover the bottom of the flask. Cells were cultured at 37 °C in a 5% CO incubator. Fresh medium was replaced every 2 days. The culture flask was regularly observed under a microscope, and cells were passaged or cryopreserved when they reached approximately 70% confluence.

1.2.2.1 Establishment of Hyperlipidemic BRL Cell Model BRL cells were inoculated into 50 mL cell culture flasks and cultured in high-glucose DMEM medium containing 10% fetal bovine serum at 37 °C in a 5% CO incubator. After cells adhered and covered the bottom of the flask, they were digested with trypsin, counted, and passaged. Cells were inoculated into 6-well plates at approximately 1.5×10 cells per well. When cells reached 80% confluence, the medium was replaced with fresh medium containing 50% fetal bovine serum, and cells were cultured for 24–48 h until intracellular lipid droplets or foam-like deposits appeared, indicating successful establishment of the hyperlipidemic hepatocyte model. The resulting cells were then placed in high-glucose DMEM medium containing 0.1% fetal bovine serum and allowed to rest for 24 h before subsequent treatment.

1.2.2.2 Experimental Grouping Cells were cultured in 6-well plates and divided into four groups with six replicates per group. The groups were as follows:

- **Normal control group**: Each well received 2.9 mL of high-glucose DMEM medium containing 10% fetal bovine serum. After 24 h, the medium was replaced, and cells were cultured for an additional 48 h, then placed in high-glucose DMEM medium containing 0.1% fetal bovine serum for 24 h, followed by the addition of 100 L of culture medium for 24 h.
- **Alfalfa saponin group**: Each well received 2.9 mL of high-glucose DMEM medium containing 10% fetal bovine serum. After 24 h, the medium was replaced, and cells were cultured for an additional 48 h, then placed in high-glucose DMEM medium containing 0.1% fetal bovine serum for 24 h, followed by the addition of 100 L of 300 g/mL alfalfa saponin solution (final concentration 100 g/mL) for 24 h.
- **Hyperlipidemic model group**: Each well received 2.9 mL of high-glucose DMEM medium containing 10% fetal bovine serum for 24 h, followed by high-glucose DMEM medium containing 50% fetal bovine serum for 48 h, then placed in high-glucose DMEM medium containing 0.1% fetal bovine serum for 24 h, followed by the addition of 100 L of culture medium for 24 h.
- **Hyperlipidemic saponin group**: Each well received 2.9 mL of high-glucose DMEM medium containing 10% fetal bovine serum for 24 h, followed by high-glucose DMEM medium containing 50% fetal bovine serum for 48 h, then placed in high-glucose DMEM medium containing 0.1% fetal bovine serum for 24 h, followed by the addition of 100 L of 300 g/mL alfalfa saponin solution (final concentration 100 g/mL) for 24 h.

1.2.3 Determination of Alfalfa Saponin Effects on BRL Cell Activity by MTT Assay

The procedure was as follows: (1) Digest logarithmic-phase cells with trypsin, terminate digestion, centrifuge to collect cells, and prepare a cell suspension adjusted to a concentration of 2×10 cells/mL; (2) After preparing the cell suspension, mix gently and add 100 L to each well of a 96-well plate, resulting in a cell density of 2,000 cells/well (edge wells were filled with sterile phosphate-buffered saline). Mix every six wells to ensure identical cell density across all wells; (3) Place the inoculated cell culture plate in the incubator. After 24 h of culture, add 50 L of alfalfa saponins at five gradient concentrations (final concentrations: 0 [control], 50, 100, 200, and 250 g/ L), with six replicate wells per concentration; (4) Incubate at 37 °C with 5% CO for 24 h and observe drug effects under an inverted microscope; (5) Add 10 L of MTT solution (5 mg/mL, i.e., 0.5% MTT) to each well and continue culturing for 4 h; (6) Centrifuge at 3,000 r/min for 5 min, carefully remove the supernatant without disturbing the crystals, add 150 L of dimethyl sulfoxide to each well, and shake on a low-speed orbital shaker for 10 min to fully dissolve the crystals; (7) Measure the absorbance of each well at 490 nm using a microplate reader.

1.3.1 Total RNA Extraction from Rat Liver and Cells

Total RNA extraction was performed as follows: (1) Homogenate preparation. For liver tissue: Place the sample in a liquid nitrogen-pre-chilled mortar and crush large tissue pieces with a grinding rod. Take 30–50 mg of liver tissue and grind vigorously until the tissue becomes a uniform fine white powder. Transfer the ground tissue powder to a 1 mL EP tube containing Trizol. For BRL cells: Aspirate the supernatant from the culture dish, add 1 mL of Trizol per 10 cm^2 of culture area, pipette up and down several times, and transfer to an EP tube. (2) Incubate at room temperature for 5 min to allow complete dissolution of tissue or cells. (3) Add 200 L of chloroform and close the cap tightly. (4) Shake vigorously by hand for 15 s. (5) Centrifuge at 12,000×g for 15 min at 4 °C, resulting in three distinct layers. (6) Tilt the centrifuge tube at a 45° angle and aspirate approximately 400 L of the upper aqueous phase into a new centrifuge tube, avoiding contact with the interphase and organic phase. (7) Add 0.5 mL of isopropanol per 1 mL of Trizol and invert gently to mix. (8) Incubate at room temperature for 10 min. (9) Centrifuge at 12,000×g for 10 min at 4 °C. (10) Discard the supernatant, leaving only the RNA pellet. (11) Add 1 mL of 75% ethanol per 1 mL of Trizol to wash the RNA pellet. (12) Vortex for several seconds, centrifuge at 7,500×g for 5 min at 4 °C, and discard the wash solution. (13) Air-dry or vacuum-dry for 5–10 min, avoiding over-drying which may make RNA difficult to dissolve. (14) Resuspend in 50 L of RNase-free water, pipette gently, and incubate at 55–60 °C for 10–15 min to ensure complete dissolution of RNA. (15) Store total RNA at -80 °C for subsequent experiments.

1.3.2 Determination of Total RNA Concentration

Total RNA concentration was measured using a Thermo micro-ultraviolet spectrophotometer at 260 nm, and the OD /OD ratio was recorded. Samples with results between 1.8 and 2.0 were considered acceptable.

1.3.3 Quality Detection of Total RNA

Electrophoresis results were detected using a gel imaging system from Syngene, UK.

1.3.4 Reverse Transcription of Total RNA

Reverse transcription of total RNA was performed using the Reverse Transcriptase M-MLV (RNase H) kit provided by Dalian Bao Biological Engineering Company to obtain cDNA.

1.3.5 Primer Design and Synthesis

Primers were designed using Primer 5.0 software based on rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH), ABCG5, ABCG8, and LDLR DNA sequences from GenBank. Primer sequences and parameters are shown in .

1.3.6 Fluorescent Quantitative PCR

This experiment used SYBR qPCR Mix as the fluorescent dye. After optimization, the optimal reaction system was determined as follows: SYBR qPCR Mix 5 L, diethyl pyrocarbonate (DEPC)-treated water 3.8 L, template cDNA 1 L, and 0.1 L each of forward and reverse primers, for a total reaction volume of 10 L. The fluorescent quantitative PCR instrument was programmed as follows: pre-denaturation at 95 °C for 2 min; 40 cycles of denaturation at 95 °C for 15 s, annealing at 60 °C for 20 s, and extension at 72 °C for 30 s; and hold at 20 °C for 10 min. Relative quantification was performed using the 2^(-ΔΔCt) method.

1.4 Statistical Analysis

Experimental data were analyzed using SPSS 13.0 statistical software for one-way ANOVA, with Duncan' s multiple comparison test for inter-group comparisons. Differences were considered significant at $P<0.05$. Results are expressed as "mean ± standard deviation."

2.1 Effects of Alfalfa Saponins on Rat Serum Indices

As shown in , compared with the normal control group, serum TG, TC, and LDL-C contents in the hyperlipidemic model group were significantly increased ($P<0.05$). Serum TG, TC, and HDL contents in the alfalfa saponin group showed slight increases, but these differences were not statistically significant ($P>0.05$). Compared with the hyperlipidemic model group, serum TG content in the hyperlipidemic saponin group decreased significantly ($P<0.05$) by 17.29%, serum TC content decreased significantly ($P<0.05$) by 12.20%, serum HDL content showed no significant change ($P>0.05$), and serum LDL-C content decreased significantly ($P<0.05$) by 21.95%.

2.2 Effects of Alfalfa Saponins on Hepatic mRNA Expressions of LDLR, ABCG5, and ABCG8 in Rats

As shown in , compared with the normal control group, mRNA expressions of LDLR, ABCG5, and ABCG8 in the alfalfa saponin group increased by 31.11-fold, 6.77-fold, and 7.26-fold, respectively, with significant differences ($P<0.05$). In the hyperlipidemic model and hyperlipidemic saponin groups, LDLR mRNA expression increased by 6.57-fold and 4.47-fold, respectively, reaching significant differences ($P<0.05$); ABCG5 mRNA expression increased by 2.32-fold and 4.37-fold, respectively, reaching significant differences ($P<0.05$); and ABCG8 mRNA expression increased by 2.57-fold and 3.40-fold, respectively, with significant differences ($P<0.05$). Compared with the hyperlipidemic model group, LDLR mRNA expression in the hyperlipidemic saponin group decreased slightly, but the difference was not significant ($P>0.05$); ABCG5 and ABCG8 mRNA expressions increased to varying degrees, with significant differences ($P<0.05$).

2.3 Effects of Alfalfa Saponins on BRL Cell Activity

As shown in , when the final concentration of alfalfa saponins was 50 or 100 g/mL, BRL cell activity showed no significant difference compared with the control group (P>0.05). However, at concentrations of 200 and 250 g/mL, BRL cell activity increased significantly compared with the control group (P<0.05).

2.4 Effects of Alfalfa Saponins on mRNA Expressions of LDLR, ABCG5, and ABCG8 in BRL Cells

As shown in , compared with the normal control group, LDLR mRNA expression in the alfalfa saponin group increased by 3.35-fold, with a significant difference (P<0.05); ABCG5 and ABCG8 mRNA expressions increased to 1.75-fold and 3.27-fold of the control group, respectively, with significant differences (P<0.05). LDLR mRNA expression in the hyperlipidemic cell group decreased, but the difference was not significant (P>0.05); LDLR mRNA expression in the hyperlipidemic saponin group increased slightly, but the difference was not significant (P>0.05). ABCG5 and ABCG8 mRNA expressions showed no significant differences between the hyperlipidemic cell and hyperlipidemic saponin groups (P>0.05). Compared with the hyperlipidemic cell group, LDLR mRNA expression in the hyperlipidemic saponin group increased, but the difference was not significant (P>0.05); ABCG5 and ABCG8 mRNA expressions in the hyperlipidemic saponin group decreased to varying degrees, but the differences were not significant (P>0.05).

3.1 Effects of Alfalfa Saponins on Rat Serum Indices

Blood lipids are the collective term for various lipids in blood, including cholesterol, TG, phospholipids (PL), and free fatty acids. Hyperlipidemia refers to the condition where one or more plasma lipids are elevated above normal levels due to abnormal lipid metabolism or transport. Clinically, hyperlipidemia is classified into three categories: hypercholesterolemia (elevated TC), hypertriglyceridemia (elevated TG), and mixed hyperlipidemia (elevated both TC and TG). Therefore, TC and TG are important indicators for evaluating hyperlipidemia. Hyperlipidemia is widely recognized as a major risk factor for atherosclerosis and other cardiovascular diseases. Numerous studies have shown that alfalfa saponins can reduce blood lipid levels and exhibit good anti-atherosclerotic effects. Yu et al. reported that alfalfa saponins significantly reduced blood TC, TG, and LDL-C contents in hyperlipidemic mice. Malinow et al. found that alfalfa saponins reduced serum TC content without altering high-density lipoprotein content and increased excretion of bile acids and neutral cholesterol. The present study showed that supplementation with alfalfa saponins significantly reduced serum TG, TC, and LDL-C contents in hyperlipidemic rats, consistent with previous research findings.

3.2 Effects of Alfalfa Saponins on BRL Cell Activity

Alfalfa saponins have hemolytic effects; injection of saponin aqueous solution into blood can cause red blood cell lysis even at low concentrations. This hemolytic effect is generally believed to be related to the interaction between saponins and cholesterol in red blood cell membranes. This study used the MTT assay to detect the effects of alfalfa saponins on BRL cell growth to determine whether alfalfa saponins are toxic to these cells and to establish the appropriate concentration for cell culture experiments, providing a foundation for subsequent studies. The results showed that alfalfa saponin concentrations below 100 g/mL had no effect on BRL cell activity; therefore, 100 g/mL was selected as the concentration for alfalfa saponin supplementation in cell culture experiments.

3.3 Effects of Alfalfa Saponins on Cholesterol Clearance and Transport

Previous research on the cholesterol-lowering mechanism of plant saponins has focused primarily on physicochemical aspects, specifically the formation of insoluble complexes between saponins and cholesterol in the intestinal tract, with few and unsystematic studies at the molecular level. Wang et al. suggested that the cholesterol-lowering effect of alfalfa saponins in hyperlipidemic rats might be related to inhibiting the transcription and expression of acetyl-CoA acetyltransferase 2 (ACAT-2), reducing cholesterol absorption, and promoting the transcription and expression of cholesterol 7 -hydroxylase (CYP7A1), accelerating the conversion of cholesterol to bile acids. Yu et al. studied the effects of alfalfa saponins on cholesterol metabolism in hyperlipidemic rats and found that the activities and mRNA expressions of lipoprotein lipase (LPL) and hepatic lipase (HTGL) were significantly increased, leading to the speculation that the cholesterol-lowering effect of alfalfa saponins was caused by increased hepatic lipoprotein lipase activity. This study investigated the effects of alfalfa saponins on mRNA expressions of key genes (LDLR, ABCG5, ABCG8) in the cholesterol clearance and secretion pathways at both animal and cellular levels, exploring the molecular mechanism by which alfalfa saponins regulate cholesterol clearance and transport.

LDLR is a transmembrane receptor located on the cell surface whose primary function is to participate in low-density lipoprotein (LDL) metabolism. Cholesterol carried by LDL in plasma is mainly cleared by hepatocyte LDLR, and the endocytosis and clearance of LDL by LDLR is the most critical step in LDL metabolism. The LDLR pathway plays a very important role in reducing serum cholesterol content, regulating low-density lipoprotein metabolism, and maintaining normal blood cholesterol levels. Nearly two-thirds of LDL-C in serum is cleared through this endocytic pathway, and the amount of LDL-C uptake depends on the quantity and activity of LDLR; therefore, LDLR plays an important role in maintaining plasma LDL-C and cholesterol levels. Numerous studies have shown that active plant compounds can regulate cholesterol con-

tent in the body by modulating LDLR mRNA expression. Luo et al. reported that curcumin significantly reduced blood cholesterol content, possibly by significantly increasing the quantity and activity of LDLR. Dou further investigated this mechanism and found that curcumin could promote LDLR expression by activating the sterol regulatory element (SRE) or by reversing the inhibition of the sterol regulatory element-binding protein (SREBP) pathway by insulin-induced gene 2 (Insig2). Liu et al. studied the effects of Dahuai mixture on LDL uptake by rat organs and cholesterol excretion, finding that Dahuai mixture could significantly enhance organ uptake of LDL and effectively promote cholesterol excretion from the body. The present study showed that supplementation with alfalfa saponins increased hepatic LDLR mRNA expression in both normal and hyperlipidemic rats by several-fold; in normal BRL cells, LDLR mRNA expression increased, with results similar to those from normal rat experiments, while alfalfa saponins had little effect on LDLR mRNA expression in hyperlipidemic BRL cells. This suggests that alfalfa saponins can accelerate the uptake and clearance of cholesterol in normal hepatocytes by regulating LDLR mRNA expression.

ABCG5 and ABCG8 are new members of the G subfamily of the ABC superfamily. ABCG5 and ABCG8 are key proteins for cholesterol entry into bile. Increased expression of these proteins enhances hepatic cholesterol transport, selectively increases neutral cholesterol excretion, and causes compensatory increases in cholesterol synthesis. Blocking these genes significantly increases plasma and hepatic cholesterol responses to dietary cholesterol. ABCG5 and ABCG8 expressed in the liver play a decisive role in biliary cholesterol transport. Yu et al. found that in ABCG5 and ABCG8 knockout mice, biliary cholesterol secretion decreased dramatically, while in ABCG5 and ABCG8 transgenic mice, biliary cholesterol content and fecal neutral cholesterol content increased significantly. The present study showed that supplementation with alfalfa saponins increased hepatic ABCG5 and ABCG8 mRNA expressions in both normal and hyperlipidemic rats by several-fold; in normal BRL cells, ABCG5 and ABCG8 mRNA expressions increased, consistent with results from normal rat experiments, while alfalfa saponins had little effect on ABCG5 and ABCG8 mRNA expressions in hyperlipidemic BRL cells. This suggests that alfalfa saponins can increase the transport of endogenous cholesterol in normal hepatocytes by regulating ABCG5 and ABCG8 mRNA expressions, thereby reducing cholesterol content. In this study, alfalfa saponins significantly affected hepatic mRNA expressions of LDLR, ABCG5, and ABCG8 in hyperlipidemic rats but had no significant effects on gene expressions in hyperlipidemic BRL cells, suggesting that the regulation of hepatic cholesterol clearance and transport by alfalfa saponins in hyperlipidemic rats may be compensatory rather than direct. The specific mechanism requires further investigation.

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Note: Figure translations are in progress. See original paper for figures.