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# Daily intake of *Lactobacillus paracasei* (*Lacticaseibacillus paracasei*) TISTR2593 ameliorated dyslipidemia and hepatic steatosis in rats fed on high-cholesterol diets by modulating lipid metabolism

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**ABSTRACT**: Dietary probiotic supplement has been documented to effectively ameliorate hyperlipidemia and its related diseases. Therefore, we aimed to study the dietary effect of *Lactobacillus paracasei* TISTR2593 (*Lp*) supplement on a high-cholesterol diet (HCD)-induced hyperlipidemia and liver steatosis in rats, and to explore the possible underlying mechanisms. The experiments were performed in 4 groups of rats, the control (receiving standard diet), and 3 treated groups: HC, HC plus *Lp* ($2 \times 10^8$ cfu/ml/day), and HC plus simvastatin (40 mg/kg/day). After 8 weeks of treatment, blood and liver were collected for biochemical analysis and histopathological studies. The hepatic expressions of proteins involved in lipid synthesis and uptake were analyzed by Western blotting. The oral administration of *Lp* lowered the serum levels of total cholesterol, triglycerides, LDL-C, alanine aminotransferase (ALT), and aspartate aminotransferase (AST), and increased serum HDL-C level in HCD-fed rats. Additionally, this probiotic reduced hepatic triglycerides and cholesterol contents and improved histological characteristics of hepatic steatosis in HCD-fed rats. Western blot analysis showed that the hepatic expressions of sterol regulatory element binding protein (SREBP)-1c, SREBP2, fatty acid synthase (FAS), stearoyl-CoA desaturase (SCD)-1, 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), and proprotein convertase subtilisin/kexin type 9 (PCSK9) were downregulated; while the expression of low-density lipoprotein receptor (LDLR) was upregulated in the HC plus *Lp*-treated group. These results indicated that daily consumption of *Lp* alleviated HCD-induced hyperlipidemia and hepatic steatosis in part by downregulating proteins involving in lipogenesis and cholesterogenesis and modulating PCSK9- and LDLR-mediated hepatic LDL-C uptake.

**KEYWORDS**: *Lactobacillus paracasei*, *Lacticaseibacillus paracasei*, vascular damage, fatty liver, lipid metabolism, hyperlipidemia

## INTRODUCTION

Hyperlipidemia is a disorder of lipid metabolism characterized by elevated levels of circulating cholesterol, triglyceride, and low-density lipoprotein cholesterol (LDL-C) and is a well-established risk factor of various diseases including non-alcoholic fatty liver disease (NAFLD) [1]. The hallmark of NAFLD is the overabundance of fat storage in the hepatocytes, mostly in the form of triglycerides. Steatosis, the early stage of fatty liver disease, could progress to serious complications; for example, liver damage, inflammation, and elevated risk of hepatocellular carcinoma [2, 3]. Therefore, the prevention of NAFLD progression must be fully considered on its association with hyperlipidemia.

Liver is the major organ responsible for maintaining lipid homeostasis by coordinating interactions between lipid synthesis, uptake, and excretion [4, 5]. The expressions of rate-limiting lipogenic and cholesterogenic genes in the liver are controlled by crucial transcription factors: sterol regulatory element binding protein-1c (SREBP-1c), and sterol regulatory element binding protein 2 (SREBP2) [6–8]. Numerous studies showed that both lipogenic and cholesterogenic pathways were often dysregulated in hyperlipidemia and NAFLD [6, 7]. Therefore, studying lipid metabolism is a viable therapeutic approach for treating hyperlipidemia and related disorders.

When consumed in adequate amounts, probiotics exert positive influence on health of the host through modulating metabolism, gut microbiome balance, and immune response; and they are widely used in fermentation of dairy products [9]. Clinical and animal studies reportedly indicated that the consumption of probiotic *Lactobacillus* species exhibited various physiological activities, including lipid-lowering, anti-

steatotic, and antioxidative effects [10–13]. *Lactobacillus paracasei*, recently renamed *Lacticaseibacillus paracasei* (*L. paracasei*), is a common lactic acid bacterial species in humans, animals, and fermented foods [14–16]. Many studies reported that administration of *L. paracasei* reduced serum cholesterol levels in hypercholesterolemic animals [17, 18] and lowered liver lipid accumulation in high fat diets-induced NAFLD rats [12] and in alcohol-induced liver diseases [19]. Recent study demonstrated that *Lactobacillus paracasei* TISTR2593 (*Lp*) reduced serum LDL-C and prevented the development of atherosclerosis in patients with hypercholesterolemia [20]. Although previous findings indicated that the *L. paracasei* strain exhibited a lipid-lowering effect, the protective effects of *Lp* against hyperlipidemia and fatty liver disease remain unclear. Therefore, the present study aimed to explore the influence of *Lp* on hyperlipidemia and NAFLD in rats fed on high-cholesterol diet (HCD). The expression of proteins involved in fatty acid and cholesterol metabolisms, such as SREBP-1c and SREBP2, as well as their target enzymes were investigated to uncover the mechanisms by which *Lp* ameliorated hyperlipidemia and NAFLD.

## MATERIALS AND METHODS

### Preparation of *Lp*

*Lp* was isolated, identified, and characterized as a potential probiotic strain by Biodiversity Research Centre, and it was kept in the TISTR culture collection. For test preparation, *Lp* was cultured in MRS (de Man, Rogosa and Sharpe) broth at 37 °C under anaerobic condition for 18–24 h, and its precipitate was collected by centrifugation at 9,000 rpm. The precipitate was washed twice with PBS, suspended in 10% maltodextrin (Qingdao Shengda Commercial & Trade Co., Ltd., Shandong, China) at a concentration of $10^{10}$ cfu/ml and then freeze dried. The freeze-dried *Lp* powder was dissolved in distilled water and administered daily by oral gavage at a concentration of $2 \times 10^8$ cfu/ml/day.

### Experimental animals

The animal protocols were approved by the Institutional Animal Care Committee of Naresuan University (ethical approval number: 630817). Male Sprague-Dawley rats weighing between 180–200 g were purchased from the Nomura Siam International Co., Ltd., Bangkok, Thailand. All the rats were housed in the animal room of the Centre for Animal Research Naresuan University for an acclimation period under a constant temperature of 22±1 °C with a 12 h light-dark cycle and free access to a standard diet including water ad libitum for one week.

After the acclimation period, the rats were randomly divided into 4 groups as follows: (1) control group (C), receiving a standard diet; (2) high cholesterol diet group (HC), receiving a HCD (standard diet + 1.5% (w/w) of cholesterol (Carlo Erba. Reagent, Cornaredo (MI), Italy), 0.37% (w/w) of cholic acid (Sigma-Aldrich, St. Louis, USA)), and the vehicle (maltodextrins in water); (3) HC plus *Lp* group (HC+LP), receiving HCD with *Lp* at $2 \times 10^8$ cfu/ml/day; and (4) HC plus simvastatin group (HC+SS), receiving a HCD with simvastatin (Sigma-Aldrich) at 40 mg/kg BW/day, as a positive control group. The rats were fed on their relevant diets for 8 weeks. The freeze-dried bacterial powder (*Lp*) and simvastatin were dissolved in distilled water and administered daily by oral gavage. The rat's food intake was recorded daily, and their body weight (BW) and length (the distance from the tip of the nose to the anus) were measured weekly to calculate body mass index (BMI), according to the method described earlier [21].

After 8 weeks, the experimental rats were fasted for 16 h and sacrificed under anesthesia. The blood was then collected via cardiac puncture [17, 22] and centrifuged for 10 min at 3000 rpm for serum separation. The liver tissues were collected, weighed, and then recorded for calculating the ratios of liver weight to body weight (LW/BW). One part of the isolated livers was fixed in a 10% neutral buffed formalin solution for histological analysis. Another part of the livers was promptly frozen using liquid nitrogen, then stored at −80 °C until required for further analysis.

### Biochemical analysis

The serum total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) levels were measured using a colorimetric assay kit (Human, Wiesbaden, Germany), according to the manufacturer's instructions. The biomarkers of the liver function, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities, in the serum were also measured by the colorimetric method using a commercial kit from the same company (Human).

### Determination of hepatic cholesterol and triglyceride contents

The liver samples were homogenized, and then the lipids were extracted using an extraction kit (Abcam, Cambridge, UK) according to the manufacturer's protocols. The liver lipid extract was suspended in 50 µl of suspension buffer and sonicated for 15 min at 37 °C. TC and TG levels in the livers were quantified using a colorimetric assay kit (Merck Millipore, Darmstadt, Germany) in accordance with the manufacturer's instructions, and the hepatic TC and TG contents were expressed as mg/g liver.

### Determination of histological changes in the liver tissues

After the liver tissues were fixed in 10% (v/v) formalin solution, they were embedded in paraffin, sliced to a

thickness of 5 µm, and then stained with hematoxylin-eosin (H&E) for determining their histological alterations. To determine the hepatic lipid accumulation, the liver was embedded in an optimal cutting temperature (OCT) compound, then sliced at a thickness of 8 µm at 20 °C, and stained with Oil Red O solution as described [23]. All tissue sections were imaged under a light microscope, and their alterations of the histological features were analyzed and quantified with the Image-J version 1.50e [23].

### Western blotting analysis

The total proteins of the liver tissues were extracted by cold RIPA buffer containing protease inhibitor cocktail (Thermo Scientific, Massachusetts, USA). Protein concentrations were measured using a micro-BCA protein assay kit. The proteins (60 µg) were separated by using SDS-PAGE, which were then transferred to polyvinylidene fluoride (PVDF) membranes (Merck Millipore). The membranes were then blocked with a 5% bovine serum albumin (BSA) in 1xTris-buffered saline solution containing 0.1% Tween-20 for 1 h, then incubated at 4 °C overnight with the following primary antibodies with dilution 1:1000: from Abcam, SREBP-1c, SREBP-2, SCD-1, and PCSK9; from Cell signaling, FAS; from Thermo Scientific, LDLR; and from Merck Millipore, HMG-CoA reductase. GAPDH from Merck Millipore was used with dilution 1:2500. After washing, the membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibody (Merck Millipore, dilution 1:5000). Protein bands were detected by the enhanced chemiluminescence (ECL) Western blotting system (BioRad laboratories, USA) and normalized by GAPDH using Image LabTM Software (BioRad laboratories).

### Statistical analysis

The data was expressed by mean ± standard error of the mean (SEM). The data was analyzed by one-way analysis of variance (ANOVA) coupled with a Tukey's post hoc test, and where appropriate with the use of the GraphPad Prism version 5. Statistical significance was accepted for $p < 0.05$.

## RESULTS

### Effect of *Lp* on HCD-induced weight gain

Table 1 revealed that there was no significant difference in the initial BW and daily food consumption during the experimental period (data not shown) among the four groups. At the end of the experimental period, the final BW, BMI, LW, and LW/BW ratio of the rats fed with HCD were significantly greater than the control group. However, the administration of *Lp* or simvastatin for 8 weeks led to a significant decrease in BW, BMI, LW and LW/BW ratio in the HCD-fed rats compared with HC Group 2 rats without probiotics or simvastatin (Table 1).

### Effect of *Lp* on serum biochemical parameters

Serum lipid profile (TC, TG, LDL-C, and HDL-C) and liver index, markers of liver injury (AST and ALT) in all groups are shown in Table 1. The serum TC, TG, LDL-C, AST, and ALT levels in the HC Group 2 were significantly increased, whereas serum HDL-C levels were significantly decreased, compared with the control group. However, compared with the HC Group 2, *Lp* or simvastatin significantly lowered serum TC, TG, LDL-C, AST, and ALT levels, and increased serum HDL-C level. These results indicated that *Lp* can reduce HCD-induced hyperlipidemia and liver injuries in rats.

### Effect of *Lp* on HCD-induced hepatic steatosis in rats

Fig. 1A depicts the histopathological sections of the liver, which were reddish-brown with sharp edges and smooth surfaces in the control group, while the ones of the HC group appeared enlarged, and yellow brown in colour. The *Lp* improved the gross anatomical appearance of the livers in the HCD-fed rats, while the livers of the HC+SS group were similar in appearance to the HC+LP group. The microscopic examination of both the H&E- and Oil Red O-stained liver sections (Fig. 1A) revealed that the hepatic lipid droplets and the percentage of the Oil Red O-stained areas (Fig. 1B) were significantly higher in the HC group than the control group, indicating the presence of hepatic steatosis in HCD-fed rats. While the treatment with *Lp* and simvastatin for 8 weeks decreased these histological characteristics of hepatic steatosis in HCD-fed rats.

To confirm the ability of the *Lp* in reducing lipid accumulation, the hepatic lipid contents were determined. The concentrations of both TG (Fig. 1C) and cholesterol (Fig. 1D) were significantly increased in the livers of HCD-fed rats, when compared with the control rats. Treatment with *Lp* and simvastatin reduced the levels of both TG (Fig. 1C) and cholesterol (Fig. 1D) in the livers of the HCD-fed rats. These results suggest that *Lp* could prevent HCD-induced hepatic lipid accumulation in rats.

### Effect of *Lp* on protein expression related to lipid metabolism

As presented in Figs. 2 and 3, the protein expression of SREBP-1C, SREBP2, FAS, SCD-1, HMGCR, and PCSK9 were significantly increased and the expression level of LDLR was decreased in the liver of HCD-fed rats when compared to those in the contro group. However, daily administration of *Lp* or simvastatin to HCD-fed rats downregulated the hepatic expression of SREBP-1C (Fig. 2B), SREBP2 (Fig. 3B), FAS (Fig. 2C), SCD-1 (Fig. 2D), HMGCR (Fig. 3C), and PCSK9 (Fig. 3D), and upregulated LDLR expression (Fig. 3E), compared to those in HC Group 2 which was only fed with HCD.

**Table 1** Effect of *Lp* on body weight, liver weight, and serum biochemical analysis in HCD-fed rats.

| Parameter | C | HC | HC+LP | HC+SS |
|---|---|---|---|---|
| Initial BW (g) | 346.9 ± 3.09 | 340.9 ± 13.21 | 342.9 ± 11.57 | 352.2 ± 11.38 |
| Final BW (g) | 592.0 ± 17.30 | 712.1 ± 16.11$^{**}$ | 622.5 ± 16.69$^{\#\#}$ | 632.1 ± 9.29$^{\#\#}$ |
| BMI (g/cm2) | 0.686 ± 0.014 | 0.775 ± 0.016$^{**}$ | 0.742 ± 0.020$^{\#\#}$ | 0.714 ± 0.014$^{\#\#}$ |
| LW (g) | 15.92 ± 0.49 | 34.00 ± 1.11$^{**}$ | 27.85 ± 1.74$^{**,\#\#}$ | 28.83 ± 1.54$^{**,\#\#}$ |
| LW/BW ratio | 0.026 ± 0.001 | 0.049 ± 0.001$^{**}$ | 0.043 ± 0.002$^{**,\#\#}$ | 0.044 ± 0.001$^{**,\#\#}$ |
| TC (mg/dl) | 54.46 ± 3.325 | 170.0 ± 13.25$^{*}$ | 116.3 ± 6.31$^{*,\#}$ | 107.7 ± 6.81$^{*,\#}$ |
| TG (mg/dl) | 84.15 ± 4.57 | 115.0 ± 6.45$^{*}$ | 69.26 ± 7.41$^{\#}$ | 90.36 ± 10.26$^{\#}$ |
| LDL-C (mg/dl) | 8.50 ± 2.47 | 125.0 ± 14.41$^{*}$ | 83.11 ± 8.34$^{*,\#}$ | 59.82 ± 7.62$^{*,\#}$ |
| HDL-C (mg/dl) | 38.40 ± 3.35 | 18.27 ± 1.04$^{*}$ | 28.07 ± 2.30$^{\#}$ | 32.48 ± 6.057$^{\#}$ |
| AST (U/l) | 82.17 ± 7.98 | 197.5 ± 17.08$^{*}$ | 107.3 ± 11.09$^{*,\#,+}$ | 59.17 ± 6.19$^{\#}$ |
| ALT (U/l) | 23.75 ± 4.95 | 128.0 ± 19.38$^{*}$ | 44.33 ± 8.71$^{\#}$ | 26.00 ± 4.25$^{\#}$ |

Results are shown as mean ± SEM for 6 rats. $^{*}p < 0.05$, $^{**}p < 0.01$ vs. C group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs. HC group, $^{+}p < 0.05$ vs. HC+SS group. BW, body weight; BMI, body mass index; LW, liver weight; LW/BW, ratio of liver weight to body weight; TC, total cholesterol; TG, triglyceride; LDL-C, low density lipoprotein-cholesterol; HDL-C, high density lipoprotein-cholesterol; AST, aspartate transaminase; ALT, aspartate aminotransferase.

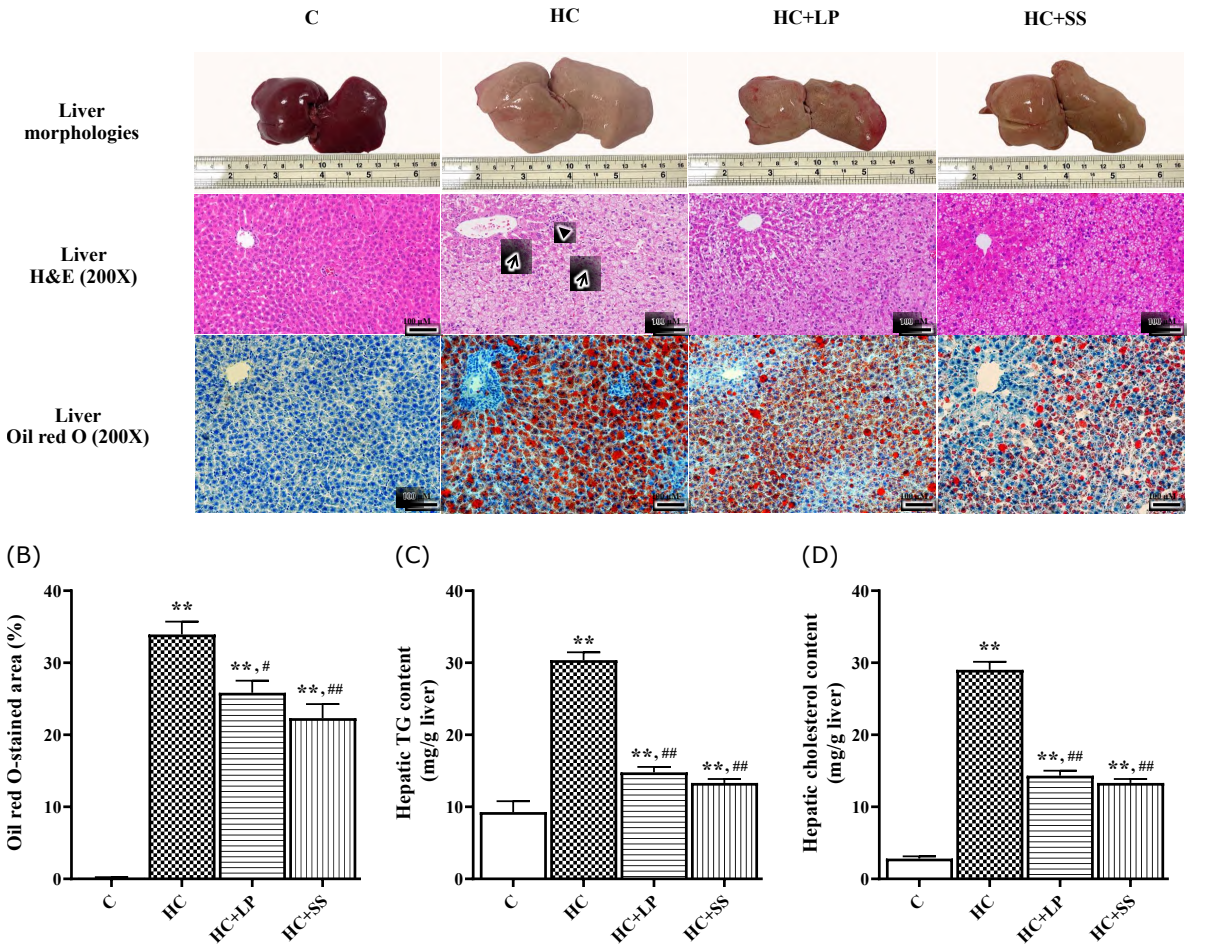


**Fig. 1** Inhibitory effect of *Lp* on HCD-induced hepatic steatosis in rats: (A) fresh liver tissues, microscopic images of H&E- and Oil Red O-stained liver tissues (magnification = ×400, scale bar = 50 µm). The arrowheads indicate inflammatory infiltrations, and the arrows indicate lipid droplets in the liver. (B) The percentage of Oil Red O-stain area; (C) hepatic TG content; (D) hepatic cholesterol content. Data is expressed as mean ± SEM. $^{**}p < 0.01$, $^{*}p < 0.05$ vs. C group; $^{\#\#}p < 0.01$, $^{\#}p < 0.05$ vs. HC group ($n = 6$ per group).

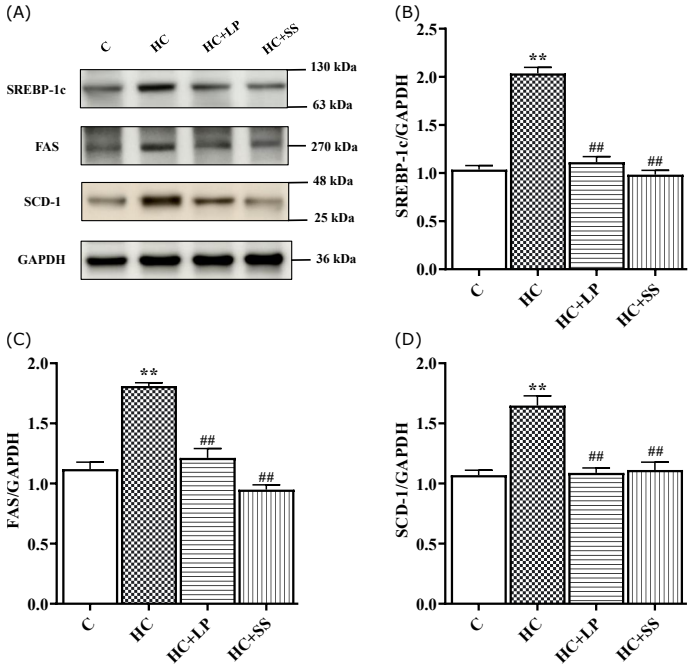


**Fig. 2** Effect of *Lp* on protein expressions of SREBP-1c, FAS and SCD-1 in the liver of HCD-induced hypercholesterolemic rats. (A) Representative bands of protein expression. Densitometry analysis of (B) SREBP-1c/ GAPDH, (C) FAS/ GAPDH, and (D) SCD-1/ GAPDH in the liver. The results are shown as mean ± SEM. $^{**}p < 0.01$, $^{*}p < 0.05$ vs. C group; $^{\#\#}p < 0.01$, $^{\#}p < 0.05$ vs. HC group ($n = 5$ per group).

## DISCUSSION

This study demonstrated that daily supplementation of *Lp* for 8 weeks reduced dyslipidemia and fatty livers in HCD-fed rats. Additionally, this probiotic strain regulated hepatic lipid metabolism in the HCD-induced hypercholesterolemia by the upregulation of LDLR protein expression, and the downregulation of the protein expression of SREBP-1c, SREBP2, and related enzymes.

Probiotic lactobacillus strains have been proposed as a potential probiotic that lower hyperlipidemia in animal and human clinical investigations [13, 18]. Previous studies demonstrated that oral administration of lactobacillus strains, such as *L. acidophilus*, *L. casei*, *L. rhamnosus*, and *L. paracasei*, effectively decreased serum levels of lipid in rats fed with HFD [13, 24, 25]. Several mechanisms of lipid-lowering effect of *lactobacillus* strain have been proposed, such as activating cholesterol binding in the intestine, promoting the excretion of bile acids through feces, altering the disturbance of intestinal microflora, and regulating specific genes and key enzymes involved in lipid metabolism [26, 27]. However, the proposed lipid-lowering mechanism for one strain may not be applied to another strain. It is well documented that simvastatin is a commonly used medication for lowering blood lipids due to its inhibitory effect on HMGCR, a rate-limiting enzyme of cholesterol synthesis pathway [7]. Therefore, it was designated as the positive control drug in this study. In the present finding, the results showed that the rats fed with a HCD for 8 weeks developed hyperlipidemia as indicated by an increase in the serum levels of TC, TG, and LDL-C, and a decrease in HDL-C level, which are consistent with previous studies [7, 22]. However, *Lp* consumption reversed these changes in HCD-fed rats, and its effect was similar to that of simvastatin treatment. These observations are consistent with previous study showing that *L. paracasei* 201 could lower serum TC, TG, and LDL-C in hypercholesterolemia rats [17]. Thus, our findings clearly document the potential of *Lp* in alleviating dyslipidemia in rats.

It is well established that overconsumption of dietary cholesterol not only causes hyperlipidemia but also results in the development of NAFLD due to changes in lipid metabolism and increased fat deposition in non-adipose tissues, particularly the liver [1, 28]. Elevated lipid influx to hepatocytes promotes an excessive accumulation of lipid, which is the earliest event triggering lipotoxicity, resulting in the dysfunction and damage of the liver [28]. Probiotics serve as a nutritional supplement for human and animal health, particularly in relation to NAFLD, which is associated with glucolipid metabolism and insulin resistance [28–30]. Several studies have provided evidence that *L. paracasei* strain can lower hepatic lipid accumulation, leading to reducing the development of fatty liver disease [12, 31, 32]. Our finding demonstrated that the hepatic lipid contents as well as serum markers of

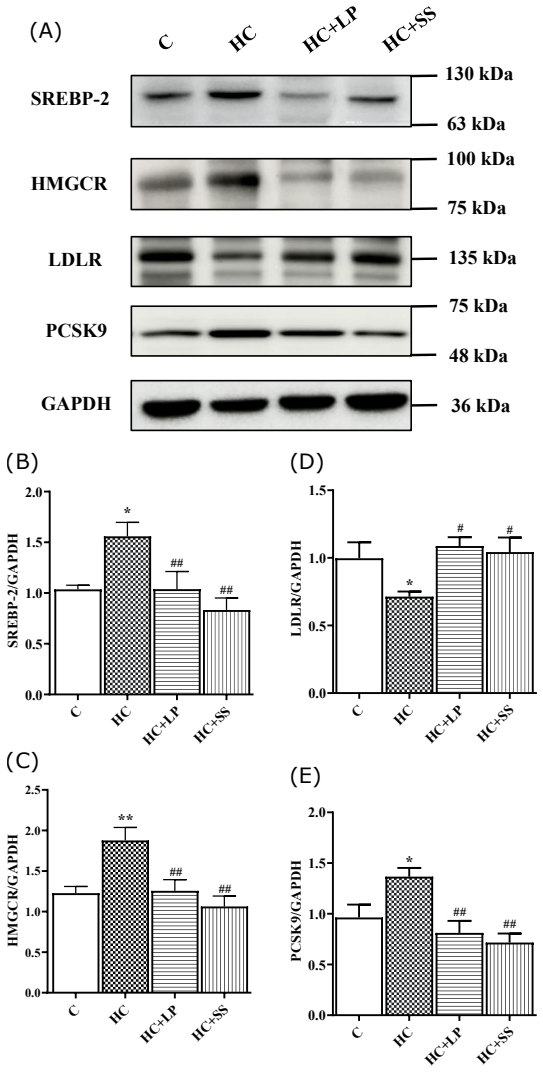


**Fig. 3** Effect of *Lp* on protein expression of SREBP2, HMGCR, PCSK9 and LDLR in the liver of HCD-induced hypercholesterolemic rats. (A) Representative bands of protein expression. Densitometry analysis of (B) SREBP2/GAPDH and (C) HMGCR/GAPDH (D) PCSK9/GAPDH and (E) LDLR/-GAPDH in the liver. The results are shown as mean ± SEM. $^{**}p < 0.01$, $^{*}p < 0.05$ vs. C group; $^{\#\#}p < 0.01$, $^{\#}p < 0.05$ vs. HC group ($n = 5$ per group)

liver damage, AST, and ALT, in *Lp*- and simvastatin-treated groups were significantly decreased as compared to HC Group 2. Both H&E and Oil Red O staining also confirmed that administration of this probiotic strain and simvastatin ameliorated HCD-induced hepatic steatosis in rats. Interestingly, simvastatin exerted greater efficacy in improving liver damage than *Lp*, although both treatments were similarly effective in reducing hepatic steatosis. Based on these findings, *Lp* might be effective in preventing and treating NAFLD and hyperlipidemia.

Generally, the concentration of circulating and hepatic lipid is related to the alteration of lipid metabolism, including intestinal absorption, endogenous synthesis, and the clearance of lipoprotein from the blood, which are regulated by various transcription factors including SREBP-1c and SREBP2 [4, 33]. SREBP-1c is responsible for the activation of genes involved in fatty acid and triglyceride synthesis such as FAS and SCD-1, which lead to the accumulation of triglyceride in liver. Fatty acid synthase (FAS) is a well-known SREBP-1c target gene and crucial enzyme required for the conversion of acetyl-CoA into palmitate. Stearoyl-CoA desaturase-1 (SCD-1) is a key enzyme involved in the desaturation of saturated fatty acids such as palmitate into monounsaturated fatty acids [6]. The upregulated expression of SREBP-1c and its target gene are directly associated with developed fatty liver and hyperlipidemia in experimental animals [6]. SREBP2 is a crucial transcription factor which activates the transcription of genes involved in mevalonate pathway, such as HMGCR, thus contributing to the regulation of endogenous cholesterol synthesis [7, 34]. The SREBP2 also regulates other genes involved in hepatic clearance of plasma LDL-C, such as LDLR and PCSK2, at the transcription level. Hepatic LDLR is a transmembrane glycoprotein that is essential for the elimination of LDL-C from the bloodstream via its binding of circulating LDL-C particles, with internalization of the LDLR-LDL complex into the hepatocyte. The LDLR activity is also regulated at the post-translational level by PCSK9, a proprotein convertase that induces internalization and degradation of LDLR in the lysosome to maintain plasma cholesterol homeostasis [4, 8, 33]. Previous studies indicated that the overexpression of SREBP2 activates the downstream targets HMGCR and PCSK9 and induces lipid accumulation and hyperlipidemia [35–37]. It has been reported that certain probiotic strains could alter the pathway of lipid synthesis and excretion, which led to reduced lipid levels. For example, The *L. paracasei* TD3 downregulated the gene expressions of both HMGCR and CYP7A1 in high fat diet (HFD)-fed rats [18]. The *L. paracasei* BY2 lowered cholesterol concentration by reducing the gene expression of both SREBP2 and HMGCR and upregulating the expression of LDLR and CYP7A1 in high-cholesterol HepG2 cell model [38]. While another study reported that the mRNA levels of both SREBP2 and HMGCR in hypercholesterolemic rats were unaffected by the *L. paracasei* 201 [17]. Thus, the cholesterol-lowering mechanisms of *L. paracasei* may be strain-specific which may be affected by several factors such as probiotic characteristics, and the change in lipid metabolism-related genes. In this study, the daily administration of *Lp* decreased the protein expression of SREBP-1c and its target enzymes, FAS and SCD-1, as well as SREBP2 and HMGCR expression, in a similar

way to simvastatin in HCD-fed rats. These results are similar to other previous *in vivo* reports, indicating that different strains of *L. paracasei*, such as *L. paracasei* JY062, *L. paracasei* 201, *L. paracasei* K56, and *L. paracasei* TD3, can regulate the lipogenic pathway at gene and protein expression levels. Studies in HFD-treated animals demonstrated that the consumption of *L. paracasei* JY062 [39] and *L. paracasei* 201 [17] could downregulate the hepatic expression of lipogenic genes, such as SREBP-1c, ACC, FAS, or SCD-1. Additionally, *L. paracasei* K56 reduced the protein levels of SREBP-1c and FAS [40], while *L. paracasei* TD3 decreased HMGCR mRNA expression [18]. This confirmed that this probiotic strain was able to reduce both fatty acid and cholesterol synthesis in the liver, thus contributing to lowered levels of serum and hepatic triglyceride and cholesterol. In addition, downregulated PCSK9 and upregulated LDLR protein expression were found in the liver of *Lp*- and simvastatin-treated rats, indicating that this probiotic strain could activate the LDLR-mediated cholesterol uptake in the liver and subsequently lower serum LDL-C level. Altogether, this finding suggested that the anti-hyperlipidemic and anti-steatotic effects of *Lp* were partly mediated by the reduction of hepatic lipid synthesis through SREBP-1c/FAS/SCD and SREBP2/HMGCR pathways, and the activation of LDLR-mediated hepatic cholesterol uptake.

In conclusion, the current study revealed that *Lp* supplementation in HCD-fed rats for 8 weeks was effective in lowering hyperlipidemia and NAFLD. The potential mechanism of its lipid-lowering effect might be related to decreasing hepatic triglyceride synthesis through the SREBP-1c/FAS/SCD signaling pathway, reducing hepatic cholesterol synthesis through the SREBP2/HMGCR signaling pathway, and increasing serum LDL-C clearance through the SREBP2/PCSK9/LDLR signaling pathway. This suggests that supplementation of *Lp* can be a useful strategy for preventing hyperlipidemia and NAFLD.

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## REFERENCES

1. Bedogni G, Gastaldelli A, Foschi FG (2020) Fatty liver, cardiometabolic disease and mortality. *Curr Opin Lipidol* **31**, 27–31.
2. Loomba R, Friedman SL, Shulman GI (2021) Mechanisms and disease consequences of nonalcoholic fatty liver disease. *Cell* **184**, 2537–2564.
3. Dhamija E, Paul SB, Kedia S (2019) Non-alcoholic fatty liver disease associated with hepatocellular carcinoma: An increasing concern. *Indian J Med Res* **149**, 9–17.
4. Ma S, Sun W, Gao L, Liu S (2019) Therapeutic targets of hypercholesterolemia: HMGCR and LDLR. *Diabetes Metab Syndr Obes* **12**, 1543–1553.
5. Hu N, Chen C, Wang J, Huang J, Yao D, Li C (2021) Atorvastatin ester regulates lipid metabolism in hyperlipidemia rats via the PPAR-signaling pathway and HMGCR expression in the liver. *Int J Mol Sci* **22**, 11170.
6. Pengnet S, Sumarithum P, Phongnu N, Prommaouan S, Kantip N, Phoungpetchara I, Malakul W (2022) Naringin attenuates fructose-induced NAFLD progression in rats through reducing endogenous triglyceride synthesis and activating the Nrf2/HO-1 pathway. *Front Pharmacol* **13**, 1049818.
7. Namwong A, Kumphune S, Seenak P, Chotima R, Nernpermpisooth N, Malakul W (2022) Pineapple fruit improves vascular endothelial dysfunction, hepatic steatosis, and cholesterol metabolism in rats fed a high-cholesterol diet. *Food Funct* **13**, 9988–9998.
8. Yang HX, Zhang M, Long SY, Tuo QH, Tian Y, Chen JX, Zhang CP, Liao DF (2020) Cholesterol in LDL receptor recycling and degradation. *Clin Chim Acta* **500**, 81–86.
9. Barzegar H, Alizadeh Behbahani B, Falah F (2021) Safety, probiotic properties, antimicrobial activity, and technological performance of *Lactobacillus* strains isolated from Iranian raw milk cheeses. *Food Sci Nutr* **9**, 4094–4107.
10. Chaiyasut C, Sivamaruthi BS, Lailerd N, Sirilun S, Khongtan S, Fukngoen P, Peerajan S, Saelee M, et al (2022) Probiotics supplementation improves intestinal permeability, obesity index and metabolic biomarkers in elderly thai subjects: A randomized controlled trial. *Foods* **11**, 268.
11. Tarrah A, Dos Santos Cruz BC, Sousa Dias R, da Silva Duarte V, Pakroo S, Licursi de Oliveira L, Gouveia Peluzio MC, Corich V, et al (2021) *Lactobacillus paracasei* DTA81, a cholesterol-lowering strain having immunomodulatory activity, reveals gut microbiota regulation capability in BALB/c mice receiving high-fat diet. *J Appl Microbiol* **131**, 1942–1957.
12. Yao F, Jia R, Huang H, Yu Y, Mei L, Bai L, Ding Y, Zheng P (2019) Effect of *Lactobacillus paracasei* N1115 and fructooligosaccharides in nonalcoholic fatty liver disease. *Arch Med Sci* **15**, 1336–1344.
13. Wu Y, Zhang Q, Ren Y, Ruan Z (2017) Effect of probiotic *Lactobacillus* on lipid profile: A systematic review and meta-analysis of randomized, controlled trials. *PLoS One* **12**, e0178868.
14. Acurcio LB, Wuyts S, de Cicco Sandes SH, Sant'anna FM, Pedroso S, Bastos RW, Dos Reis DC, Vieira AF, et al (2020) Milk fermented by *Lactobacillus paracasei* NCC 2461 (ST11) modulates the immune response and microbiota to exert its protective effects against *Salmonella typhimurium* infection in mice. *Probiotics Antimicrob Proteins* **12**, 1398–1408.
15. Yang J, Dong C, Ren F, Xie Y, Liu H, Zhang H, Jin J (2022) *Lactobacillus paracasei* M11-4 isolated from fermented rice demonstrates good antioxidant properties *in vitro* and *in vivo*. *J Sci Food Agric* **102**, 3107–3118.
16. Han DH, Kim WK, Park S, Jang YJ, Ko G (2020) *Lactobacillus paracasei* treatment modulates mRNA expres-

sion in macrophages. *Biochem Biophys Rep* **23**, 100788.
17. Yang L, Xie X, Li Y, Wu L, Fan C, Liang T, Xi Y, Yang S, et al (2021) Evaluation of the cholesterol-lowering mechanism of *Enterococcus faecium* strain 132 and *Lactobacillus paracasei* strain 201 in hypercholesterolemia rats. *Nutrients* **13**, 1982.
18. Dehkohneh A, Jafari P, Fahimi H (2019) Effects of probiotic *Lactobacillus paracasei* TD3 on moderation of cholesterol biosynthesis pathway in rats. *Iran J Basic Med Sci* **22**, 1004–1009.
19. Tsai YS, Lin SW, Chen YL, Chen CC (2020) Effect of probiotics *Lactobacillus paracasei* GKS6, *L. plantarum* GKM3, and *L. rhamnosus* GKLC1 on alleviating alcohol-induced alcoholic liver disease in a mouse model. *Nutr Res Pract* **14**, 299–308.
20. Khongrum J, Yingthongchai P, Boonyapranai K, Wongtanasarasin W, Aobchecy P, Tateing S, Prachansuwan A, Sitdhipol J, et al (2023) Safety and effects of *Lactobacillus paracasei* TISTR 2593 supplementation on improving cholesterol metabolism and atherosclerosis-related parameters in subjects with hypercholesterolemia: A randomized, double-blind, placebo-controlled clinical trial. *Nutrients* **15**, 661.
21. Novelli EL, Diniz YS, Galhardi CM, Ebaid GM, Rodrigues HG, Mani F, Fernandes AA, Cicogna AC, et al (2007) Anthropometrical parameters and markers of obesity in rats. *Lab Anim* **41**, 111–119.
22. Prommaouan S, Nernpermpisooth N, Pengnet S, Paseephol T, Tunsophon S, Malakul W (2022) Wax apple (*Syzygium samarangense*) fruit extract ameliorates endothelial dysfunction and liver damage in high cholesterol diet-fed rats. *J Tradit Complementary Med* **12**, 584–593.
23. Malakul W, Seenak P, Jumroon N, Arikit S, Kumphune S, Nernpermpisooth N (2022) Novel coconut vinegar attenuates hepatic and vascular oxidative stress in rats fed a high-cholesterol diet. *Front Nutr* **9**, 835278.
24. Wang LC, Pan TM, Tsai TY (2018) Lactic acid bacteria-fermented product of green tea and Houttuynia cordata leaves exerts anti-adipogenic and anti-obesity effects. *J Food Drug Anal* **26**, 973–984.
25. Li Z, Jin H, Oh SY, Ji GE (2016) Anti-obese effects of two *Lactobacilli* and two *Bifidobacteria* on ICR mice fed on a high fat diet. *Biochem Biophys Res Commun* **480**, 222–227.
26. Reis SA, Conceicao LL, Rosa DD, Siqueira NP, Peluzio MCG (2017) Mechanisms responsible for the hypocholesterolaemic effect of regular consumption of probiotics. *Nutr Res Rev* **30**, 36–49.
27. Wang G, Huang W, Xia Y, Xiong Z, Ai L (2019) Cholesterol-lowering potentials of *Lactobacillus* strain overexpression of bile salt hydrolase on high cholesterol diet-induced hypercholesterolemic mice. *Food Funct* **10**, 1684–1695.
28. Rodríguez-Pastén A, Fernández-Martínez E, Pérez-Hernández N, Soria-Jasso LE, Cariño-Cortés R (2023) Prebiotics and probiotics: Effects on dyslipidemia and NAFLD/NASH and the associated mechanisms of action. *Curr Pharm Biotechnol* **24**, 633–646.
29. Chonghan Y, Foongsawat N, Tulathon C, Sunthornthummas S, Rangsiruji A, Sarawaneeyaruk S, Sukontasing S, Insian K, et al (2024) Development of probiotic-enriched dog snacks for improved canine digestive health. *ScienceAsia* **50**, 2024106.
30. Qi W-w, Liu Y, Liu P-f, Yi M, Zhang C, Zhang S, Zhong L-Y (2024) 11β- hydroxysteroid dehydrogenase type 1 selective inhibitor alleviates insulin resistance and non-alcoholic fatty liver disease in db/db mice. *ScienceAsia* **50**, 2024076.
31. Ye H, Li Q, Zhang Z, Sun M, Zhao C, Zhang T (2017) Effect of a novel potential probiotic *Lactobacillus paracasei* Jlus66 isolated from fermented milk on nonalcoholic fatty liver in rats. *Food Funct* **8**, 4539–4546.
32. Lee NY, Shin MJ, Youn GS, Yoon SJ, Choi YR, Kim HS, Gupta H, Han SH, et al (2021) *Lactobacillus* attenuates progression of nonalcoholic fatty liver disease by lowering cholesterol and steatosis. *Clin Mol Hepatol* **27**, 110–124.
33. Guo S, Xia XD, Gu HM, Zhang DW (2020) Proprotein convertase subtilisin/kexin-Type 9 and lipid metabolism. *Adv Exp Med Biol* **1276**, 137–156.
34. Shi J, Li R, Liu Y, Lu H, Yu L, Zhang F (2019) Shuangyu Tiaozhi Granule attenuates hypercholesterolemia through the reduction of cholesterol synthesis in rat fed a high cholesterol diet. *BioMed Res Int* **2019**, 4805926.
35. Lebeau PF, Byun JH, Platko K, Saliba P, Sguazzin M, MacDonald ME, Paré G, Steinberg GR, et al (2022) Caffeine blocks SREBP2-induced hepatic PCSK9 expression to enhance LDLR-mediated cholesterol clearance. *Nat Commun* **13**, 770.
36. Liang J, Li W, Liu H, Li X, Yuan C, Zou W, Qu L (2022) Di'ao Xinxuekang capsule improves the anti-atherosclerotic effect of atorvastatin by downregulating the SREBP2/PCSK9 signalling pathway. *Front Pharmacol* **13**, 857092.
37. Gai Y, Li Y, Xu Z, Chen J (2019) Pseudoprotodioscin inhibits SREBPs and microRNA 33a/b levels and reduces the gene expression regarding the synthesis of cholesterol and triglycerides. *Fitoterapia* **139**, 104393.
38. Cui HL, Li MF, Liu SY, Yu M, Lou WY (2022) Regulation of hepatocellular cholesterol metabolism by *Lactobacillus paracasei* BY2 and its embedding delivery. *Probiotics Antimicrob Proteins* **16**, 181–195.
39. Su Y, Ren J, Zhang J, Zheng J, Zhang Q, Tian Y, Zhang Y, Jiang Y, et al (2024) *Lactobacillus paracasei* JY062 alleviates glucolipid metabolism disorders via the adipoinsular axis and gut microbiota. *Nutrients* **16**, 267.
40. Zhang W, Jia X, Xu Y, Xie Q, Zhu M, Zhao Z, Hao J, Li H, et al (2023) Effects of Coix seed extract, *Lactobacillus paracasei* K56, and their combination on the glycolipid metabolism in obese mice. *J Food Sci* **88**, 1197–1213.