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ORIGINAL ARTICLE

# Hypocholesterolaemic action of *Lactobacillus plantarum* VJC38 in rats fed a cholesterol-enriched diet

Venkata Satyanarayana Nallala [1] · Kadirvelu Jeevaratnam [1]

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## Abstract

This study was conducted to evaluate hypocholesterolaemic activity of probiotic strains *Lactobacillus plantarum* VJC38 and VJI21 in Wistar albino rats fed a cholesterol-enriched diet. The experimental animals were divided into five groups ($n = 6$) viz., normal diet control group (NDC), hypercholesterolaemic diet (HD) control group (HDC), HD supplemented with $3 \times 10^8$ CFU/ml of *L. plantarum* VJC38 group (HD-C38), HD supplemented with $3 \times 10^8$ CFU/ml of *L. plantarum* VJI21 group (HD-I21), and HD supplemented with $3 \times 10^8$ CFU/ml of *L. rhamnosus* GG group (HD-GG) as positive control. Animals were administered bacterial culture by oral gavage once daily for 45 days. After trial, animals were sacrificed and blood samples were collected. Serum total cholesterol (T-CHO), triglyceride (TG), high-density lipoprotein (HDL) cholesterol, glucose, glutamyl pyruvate transaminase (GPT), and glutamyl oxaloacetate transaminase (GOT) levels were determined. Serum low-density lipoprotein (LDL) cholesterol levels were estimated using the Friedewald's equation. Liver and fecal lipid contents and fecal cholic acids were measured. Serum T-CHO levels were significantly decreased by 15.6 and 17.4% in the HD-GG and HD-C38 groups, respectively, but not in the HD-I21 group compared with HDC group ($P < 0.05$). HD-GG and HD-C38 groups showed 26.3 and 27.2% reduction in serum LDL cholesterol, respectively when compared with HDC group ($P < 0.05$). Serum LDL cholesterol levels in HD-I21 group were not significantly different from HDC group. Serum TG levels in the HD-GG and HD-C38 were decreased by 14.2 and 22.8%, respectively compared with HDC group ($P < 0.05$). Liver T-CHO and TG levels in the HD-GG, HD-C38, and HD-I21 were reduced significantly compared with the HDC group ($P < 0.05$). Atherogenic coefficient values of HD-GG, HD-C38, and HD-21 were significantly decreased compared with HDC group ($P < 0.05$). Serum GPT levels in the HD-GG, HD-C38, and HD-I21 were decreased by 20.6, 10.9, and 20.6%, respectively, vs. the HDC group. Serum GOT levels were not significantly different among the groups. Serum glucose levels were significantly low in HD-GG, HD-C38, and HD-I21 compared with HDC group ($P < 0.05$). Fecal cholesterol and cholic acid levels were significantly higher in the HD-C38 and HD-GG groups than other groups ($P < 0.05$). This study suggests that *L. plantarum* VJC38 exhibits hypocholesterolaemic effect through hydrolysis of conjugated bile acids in the small intestine and excretion of cholesterol in feces. *Lactobacillus plantarum* VJC38 could be used as a potential cholesterol-lowering probiotic after validation of the hypocholesterolaemic activity in placebo-controlled human clinical trials.

**Keywords** Hypocholesterolaemic activity · *Lactobacillus plantarum* · Probiotic · Rats

## Introduction

Coronary heart disease (CHD) is a leading cause of death around the world (Liu 2007). High total cholesterol (T-CHO) and low-density lipoprotein (LDL) cholesterol levels in the serum are major risk factors for CHD (Rosengren et al. 1997). Although several cholesterol-lowering drugs have been developed, they are expensive and can have severe side effects, particularly in a long-term therapeutic use (Elis and Lishner 2012). Thus, a number of studies have been conducted on different dietary ways of reducing serum T-CHO including consumption of food containing low cholesterol, dietary fiber, soy protein, plant sterols, or probiotics with cholesterol-lowering activity (Jenkins et al. 2010; Nijjar et al. 2010; Ooi et al. 2010; Gupta et al. 2011). In recent years, cholesterol-lowering activity of probiotic lactic acid bacteria (LAB), particularly lactobacilli, has gained more interest in public as a

✉ Kadirvelu Jeevaratnam
jeevskj@gmail.com

[1] Department of Biochemistry and Molecular Biology, School of Life Sciences, Pondicherry University, Kalapet, Puducherry 605014, India

safe and cost-effective method to reduce high cholesterol levels in the serum.

Probiotics are defined as "live microorganisms which, when administrated in adequate amounts, confer a health benefit on the host" (FAO/WHO 2002). One of the most important beneficial effects related to probiotics is that their ability to reduce serum cholesterol levels. Several studies have reported cholesterol-lowering activity of different *Lactobacillus* cultures (Fukushima and Nakano 1995; Taranto et al. 1998; Usman and Hosono 2000; Liong and Shah 2006; Le and Yang 2018). Many authors have proposed several mechanisms to explain hypocholesterolaemic effect of probiotics based on in vitro experimental results. These include assimilation of cholesterol by probiotics (Bottazzi et al. 1986), cholesterol binding to cell wall of probiotics (Hosono and Tonooka 1995), enzymatic degradation of bile salts by bile salt hydrolases (BSH) of probiotics (Begley et al. 2006; Lambert et al. 2008), co-precipitation of cholesterol with deconjugated bile (Liong and Shah 2006), and incorporation of cholesterol into cell membrane during growth of probiotics (Lye et al. 2010). However, a very few reports are available on the use of probiotics to reduce serum cholesterol levels in rats or other animal models and mechanisms to reduce serum cholesterol levels by LAB are not yet completely understood.

It has been shown that BSH activity of probiotics is the primary mode of hypercholesterolaemic action. BSH activity of probiotics deconjugates bile salts in the small intestine. Deconjugated bile salts are poorly absorbed from the small intestine and excreted more rapidly than the conjugated bile salts. As a result, synthesis of bile salts from cholesterol increases in the liver, which in turn reduces the T-CHO concentration in the body (DeRodas et al. 1996; Kumar et al. 2011).

*Lactobacillus plantarum* is one of the dominant species in fermented foods (Luxananil et al. 2009; Damodharan et al. 2015a), dairy products, and gastrointestinal tract (GIT) of humans and animals (Kandler and Weiss 1998). In our previous studies *L. plantarum* VJC38 and VJI21 exhibited tolerance to low pH, artificial gastric and intestinal juices, and bile salts, more adherence to the intestinal epithelial cells, BSH activity, and cholesterol-lowering activity in vitro (Nallala et al. 2017; Nallala and Jeevaratnam, 2018).

The objective of the present study was to evaluate cholesterol-lowering activity of *L. plantarum* VJC38 and VJI21 and establish their functional mechanism in rats fed a cholesterol-enriched diet.

## Materials and methods

### Bacterial strains and growth conditions

*Lactobacillus plantarum* VJC38 and VJI21, subjects of the present study, were isolated from the GIT of broiler chicken (*Gallus gallus domesticus*) and identified to the species level by partially sequencing 16S rRNA (VJC38-KP144784, VJI21-KP144785) and *dnaK* genes (VJC38-KU600225, VJI21-KX759006). Probiotic attributes of these strains were studied previously in our laboratory according to FAO/WHO guidelines (Nallala et al. 2017; Nallala and Jeevaratnam 2018). The probiotic strain *L. rhamnosus* GG (=NCDC347), used as a reference strain in the present study, was purchased from National Collection of Dairy Cultures (NCDC), National Dairy Research Institute, Karnal, India (Mandal et al. 2015). Stock cultures were stored in De man-Ragosa-Sharpe (MRS) broth (Himedia, India) with 30% glycerol at − 70 °C. Cultures were subcultured three times in MRS broth using 1% inoculums and 18-h incubation at 37 °C prior to experimental use. *L. plantarum* VJC38 strain was deposited in the Microbial Type Culture Collections (MTCC) and catalogue number MTCC 12729 was assigned to this strain.

### Experimental animals

Five-week-old male Wistar albino rats were purchased from Sri Ragavendra Enterprises, Bangalore, India, and were kept at the animal house maintained at Pondicherry University, Puducherry, India. All animals were housed two per cage and maintained on a 12-h light-dark cycle. Temperature and humidity were controlled at 20 to 25 °C and 60 to 65%, respectively. Animals were allowed free access to water and fed for the first week with a commercially prepared pellet-diet for acclimatization. Before conducting the animal trial, prior approval of the Institute's Animal Ethics Committee, Pondicherry University (PU/SLS/AH/IAEC/2015/10) was obtained and animals were maintained in accordance with the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

### Diet and experimental design

After acclimatization, animals were divided into five groups of six animals each and there were no significant differences in body weight between groups at the start of the experimental period (the average initial body weight (g) were 113 ± 16, 113 ± 16, 112 ± 17, 111 ± 18, and 113 ± 20 respectively, for the five experimental groups). One group was fed a normal diet control (NDC) and other four groups were fed hypercholesterolaemic diet (HD) for 5 weeks to induce hypercholesterolemia before the oral administration of LAB. The composition of the hypercholesterolaemic diet was (g/100 g) casein, 20; corn starch, 50; refined oil (Sunflower), 10; cholesterol, 0.50; cellulose, 3.87; vitamin mixture (AIN-93; American Institute of Nutrition, Bethesda, MD), 1.01; mineral mixture (AIN-93; American Institute of Nutrition), 4; choline chloride, 0.20; sodium cholate, 0.12; Methionine, 0.30; and sucrose, 10. After 5 weeks, HD-fed rats were divided into following

groups: HD-control (HDC); HD supplemented with $3 \times 10^8$ CFU/ml of *L. rhamnosus* GG (=NCDC347), (HD-GG); or HD supplemented with $3 \times 10^8$ CFU/ml of *L. plantarum* VJC38 (=MTCC 12729), (HD-C38); or HD supplemented with $3 \times 10^8$ CFU/ml of *L. plantarum* VJI21, (HD-I21). The dosage rate of probiotics ($3 \times 10^8$ CFU/ml) was decided based on earlier studies on hypocholesterolaemic activity of lactic acid bacteria (Damodharan et al. 2015b; Kumar et al. 2011). Bacterial cells, grown overnight at 37 °C, were pelleted at 6000×*g*, 4 °C for 10 min and washed twice with saline. After washing, cell pellet was mixed in saline to a final concentration of $3 \times 10^8$ CFU/ml and administered by oral gavage once daily for 45 days. The control NDC and HDC groups were administered saline alone.

### Sample collection

Food intake (g/rat/day) was determined by subtracting the weight of remaining food from the initial food given on the previous day, and dividing by the number of rats housed in the cage. The body weight measured once per week. Fecal samples were collected, dried, and stored at − 20 °C until analyzed. At the end of the 45-day feeding trial, rats were fasted overnight, and anesthetized by diethyl ether. Blood samples were collected immediately in sterile tubes by heart puncture. Blood samples were centrifuged (1500×*g* for 20 min at 4 °C), and the serum was separated and stored at − 20 °C until analyzed. The spleen, liver, and kidneys were removed immediately, rinsed with cold saline, and weighed. Liver samples were stored at − 80 °C after freezing in liquid nitrogen for later estimation of lipids.

### Serum biochemical analysis

Serum T-CHO, TG,HDL cholesterol, glucose, glutamyl pyruvate transaminase (GPT), and glutamyl oxaloacetate transaminase (GOT) levels were determined by using commercial assay kits (Span Diagnostic Ltd., Gujarat, India). Serum LDL cholesterol levels were estimated using the Friedewald's equation (Kumar et al. 2011), and atherogenic coefficient was calculated by the following formula, (T-CHO – HDL cholesterol)/HDL cholesterol (Guo and Li 2013).

### Analysis of liver and fecal lipid contents

Lipids from the liver and feces were extracted according to the Folch method (Walker 2009). One gram of sample was homogenized in 20 ml of chloroform-methanol (2:1) mixture. After dispersion, the homogenate was incubated at room temperature for 20 min with agitation. The homogenate was filtered and the liquid phase was washed with 4 ml of saline. The mixture was centrifuged at low speed (2000×*g*) to separate the two phases. The lower phase containing lipids was evaporated under vacuum in a rotary evaporator (Buchi, Switzerland). T-CHO and TG levels in the extracted lipid fraction were estimated using commercial assay kits mentioned above.

### Analysis of fecal cholic acid

Fecal cholic acid levels were measured following the method of Kumar et al. (2011). Dried feces (0.1 g) were extracted twice with 3.5 ml of ethanol at 80 °C for 1 h. After extraction, the ethanol phase was evaporated in rotary evaporator at 50 °C and the residue was dissolved in 2.5 ml ethanol. The alcoholic extract was dried and the residue was dissolved in 2.5 ml of 60% glacial acetic acid. Then, 1 ml of resulting solution was aliquoted into two test tubes and 6 ml of 8 M sulfuric acid was added to each test tube. After that, 1 ml of 1% furfural solution in 60% glacial acetic acid was added to one of the tubes and 1 ml of 60% glacial acetic acid was added to other tube that served as a blank. The contents were mixed by vortexing before incubating at 67 °C for 15 min and allowed to cool at room temperature. Cholic acid concentration in the resulting solution was estimated by measuring absorbance at 610 nm and comparing with cholic acid standards. Two cholic acid standards (0.25 and 0.5 mg) were prepared and treated exactly as were the unknowns. The fecal cholic acid levels were calculated using following formula.

$$\text{Cholic acid } (\mu\text{mol/l}) = \frac{A-B}{C-D} \times \text{cholic acid } (\mu\text{mol/l})$$

*A* is the absorbance at 610 nm of the sample containing furfural; *B* is the absorbance at 610 nm of the sample blank; *C* is the absorbance at 610 nm of standard containing furfural; and *D* is the absorbance at 610 nm of standard blank.

### Statistical analysis

Data are expressed as mean ± standard deviation (SD), and statistical analysis was carried out by one-way analysis of variance (ANOVA), followed by Turkey's multiple comparison tests using SPSS 17.0 (SPSS Inc., Chicago, USA). Values of $P < 0.05$ were considered to indicate statistical significance.

## Results

### Food intake, body weight gain, and organ weight

The food intake, food efficiency ratio, body weight gain, and organ weight are shown in the Table 1. Food intake was higher in the HDC group than in HD-LAB groups and NDC group. Body weight gain of HDC control group was significantly higher than other groups and there was no significant difference in body weight gain among the NDC and HD-LAB

groups. Liver weights of NDC group were lower than other groups, but there was no significant difference in liver weights among HDC group and HD-LAB groups. Kidney and spleen weights were not significantly different among the groups (Table 1).

## Serum lipid profiles

Serum T-CHO, LDL cholesterol, TG, and glucose levels in the NDC group were significantly lower compared to the HDC group ($P < 0.05$). Serum T-CHO levels were significantly decreased by 15.6 and 17.4% in the HD-GG and HD-C38 groups, respectively when compared with HDC group ($P < 0.05$). HD-I21 group showed 4.3% reduction in serum T-CHO levels but there was no significant difference with HDC group (Fig. 1). HD-GG and HD-C38 groups showed 26.3 and 27.2% reduction in serum LDL cholesterol, when compared with HDC group ($P < 0.05$). HDL cholesterol levels in the HD-GG and HD-C38 groups were significantly higher than the HDC group ($P < 0.05$). Serum LDL cholesterol and TG levels in HD-I21 group were not significantly different from HDC group. Serum TG levels in the HD-GG and HD-C38 were decreased by 14.2 and 22.8%, respectively (Fig. 1). Serum GPT levels in the HD-GG, HD-C38, and HD-I21 were decreased by 20.6, 10.9, and 20.6%, respectively, vs. the HDC group. Serum GOT levels were not significantly different among the groups. Serum glucose levels were significantly low in HD-GG, HD-C38, and HD-I21 compared with HDC group ($P < 0.05$) (Table 2).

## Atherogenic coefficient

Figure 2 shows atherogenic coefficient values of rats fed different diets. HD-GG, HD-C38, and HD-I21 groups showed significantly lower levels of atherogenic coefficient when compared with HDC group ($P < 0.05$). Atherogenic coefficient of HD-C38 group was 36.8% lower than the control HDC group.

## Liver and fecal lipid contents

Liver T-CHO and TG levels were significantly lower in the NDC group (Table 2) compared with the HDC group ($P < 0.05$). Liver T-CHO levels in the HD-GG, HD-C38, and HD-I21 were reduced significantly by 35.2, 46.7, and 18.0%, respectively, compared with the HDC group ($P < 0.05$). Liver TG levels in the HD-GG, HD-C38, and HD-I21 were decreased by 12.1, 18.3, and 8.7%, respectively, compared with HDC group ($P < 0.05$) (Table 2). Fecal cholesterol levels were increased significantly in HD-GG and HD-C38 groups compared with control HDC group. Fecal cholesterol levels in HD-I21 group were not significantly different from HDC group (Table 2).

## Fecal cholic acid excretion

Fecal cholic acid excretion levels of rats fed different diets were shown in Fig. 3. Fecal cholic acid excretion was significantly ($P < 0.05$) higher in the HD-C38, HD-GG, and HD-I21 groups when compared with control HDC group.

# Discussion

Hypercholesterolaemia is one of the major risk factors for CHD. Reduction in serum T-CHO levels in hypercholesterolaemic patients reduces the risk of CHD. Although studies have reported several drug therapies to reduce serum cholesterol levels effectively, they are expensive and have severe side effects (Silva et al. 2006; Elis and Lishner 2012). Probiotics with cholesterol-lowering activity, especially lactobacilli, have gained more interest in public as cost-effective, natural, and safe method to reduce serum cholesterol levels. Lactobacilli

**Table 1** Body weight gain, food intake, food efficiency ratio, and organ weights of Wistar albino rats fed a normal diet, hypercholesterolaemic diet alone, or supplemented with different lactic acid bacteria strains (GG, VJC38, or VJI21)

| Component | NDC | HDC | HD-GG | HD-C38 | HD-I21 |
|---|---|---|---|---|---|
| Body weight (BW) gain (g) | 158.0 ± 5.49$^{a}$ | 192.7 ± 6.71$^{b}$ | 163.8 ± 9.75$^{a}$ | 159.5 ± 7.50$^{a}$ | 167.2 ± 9.54$^{a}$ |
| Food intake (g) | 501.0 ± 8.94$^{a}$ | 589.7 ± 9.30$^{b}$ | 551.5 ± 6.60$^{c}$ | 541.7 ± 5.28$^{c}$ | 550.0 ± 6.30$^{c}$ |
| Food efficiency ratio (%) | 31.5 ± 1.15$^{a,b}$ | 32.7 ± 1.33$^{a}$ | 29.7 ± 1.98$^{b}$ | 29.4 ± 1.16$^{b}$ | 30.41 ± 1.95$^{a,b}$ |
| Organ weight (g/100 g of BW) | | | | | |
| Liver | 2.63 ± 0.31$^{a}$ | 3.20 ± 0.45$^{a,b}$ | 3.46 ± 0.19$^{b}$ | 3.76 ± 0.40$^{b}$ | 3.61 ± 0.58$^{b}$ |
| Kidney | 0.73 ± 0.07$^{a}$ | 0.66 ± 0.04$^{a}$ | 0.70 ± 0.08$^{a}$ | 0.64 ± 0.06$^{a}$ | 0.69 ± 0.08$^{a}$ |
| Spleen | 0.40 ± 0.05$^{a}$ | 0.35 ± 0.05$^{a}$ | 0.35 ± 0.04$^{a}$ | 0.36 ± 0.04$^{a}$ | 0.36 ± 0.04$^{a}$ |

*NDC*, normal diet control; *HDC*, hypercholesterolaemic diet control; *HD-GG*, HD with *L. rhamnosus* GG NCDC347; *HD-C38*, HD with *L. plantarum* VJC38; *HD-I21*, HD with *L. plantarum* VJI21; Results are expressed as mean ± standard errors of the means; $n = 6$. Means in a row with different lowercase letters (a–c) are significantly different ($P < 0.05$)

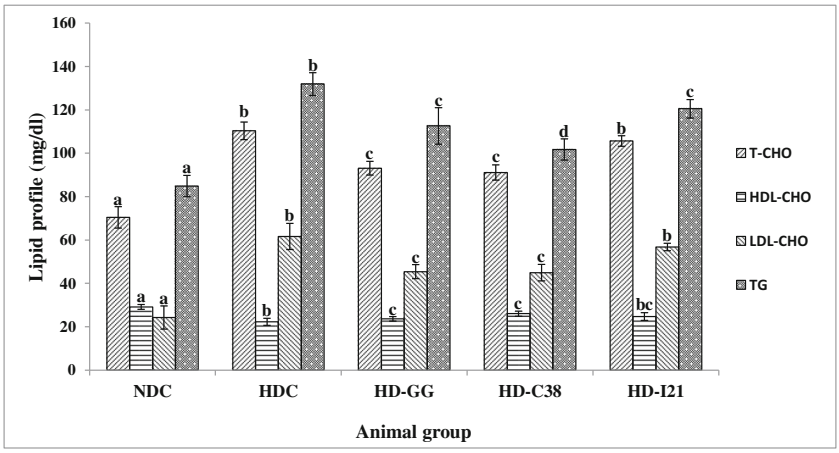


**Fig. 1** Total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides levels in serum of rats fed a normal diet (ND), hypercholesterolaemic diet (HD), or hypercholesterolaemic diet supplemented with different lactic acid bacteria strains. NDC, normal diet control; HDC, hypercholesterolaemic diet control; HD-GG, HD supplemented with *L. rhamnosus* GG; HD-C38, HD supplemented with *L. plantarum* VJC38; HD-I21, HD supplemented with *L. plantarum* VJI21. Results are expressed as means ± standard errors of the means; $n = 6$. Means within the same lipid series with different lowercase letters (a–d) are significantly different ($P < 0.05$)

are generally regarded as safe and they are common inhabitants of GIT of animals and humans (Guarner and Schaafsma 1998). Studies have reported hypocholesterolaemic effect of LAB in animal models and human subjects (Fukushima and Nakano 1995; Kumar et al. 2011; Wang et al. 2012; Guo and Li 2013; Liu et al. 2017; Kobyliak et al. 2018; Park et al. 2018). In our previous studies, *L. plantarum* VJC38 and VJI21 showed tolerance to low pH and bile salts, survivability in simulated artificial gastric and intestinal juices, adherence to the intestinal epithelial cells, BSH activity, and cholesterol-lowering activity in vitro (Nallala et al. 2017; Nallala and Jeevaratnam 2018). In this study *L. plantarum* VJC38 and VJI21, which exhibited potential probiotic properties, were selected for testing cholesterol-lowering activity in vivo in atherogenic diet-induced hypercholesterolaemic rats in comparison with reported probiotic strain *L. rhamnosus* GG as positive control. *Lactobacillus* strains were administrated to rats after feeding with cholesterol-enriched diet to treat hypercholesterolaemic condition and understand their functional mechanism. The present study showed that strain *L. plantarum* VJC38 was effective in lowering serum T-CHO, LDL cholesterol, TG, and liver T-CHO levels while increasing the fecal excretion cholesterol, compared with hypercholesterolaemic diet control group. Supplementation of VJI21 strain did not show significant reduction in serum cholesterol, LDL cholesterol, and TG levels of rats fed hypercholesterolaemic diet. The results indicated significant increase in HDL cholesterol in the groups fed GG and VJC38 strains in comparison with HDC and HD-I21 groups. The results are in accordance with the earlier studies (Bottazzi et al. 1986; Morrow et al., 2012; Wang et al. 2012), where rats fed hypercholesterolaemic diet and LAB showed significant decrease in serum T-CHO and increase in HDL cholesterol. Atherogenic coefficient value is a good predicator of heart diseases, the higher the value, the higher the risk of

**Table 2** SGPT, SGOT, serum glucose, liver total cholesterol, liver triglycerides, fecal total cholesterol, fecal triglycerides, and fecal cholic acid levels in Wistar albino rats fed a normal diet, hypercholesterolaemic diet alone, or supplemented with different lactic acid bacteria strains (GG, VJC38, or VJI21)

| Component | NDC | HDC | HD-GG | HD-C38 | HD-I21 |
|---|---|---|---|---|---|
| Serum GPT (IU/L) | $43.17 \pm 5.60^{a,b}$ | $48.62 \pm 4.36^{a}$ | $38.60 \pm 5.27^{b}$ | $43.32 \pm 7.87^{a,b}$ | $38.60 \pm 3.44^{b}$ |
| Serum GOT (IU/L) | $104.02 \pm 9.20^{a}$ | $105.49 \pm 5.08^{a}$ | $103.72 \pm 19.36^{a}$ | $96.65 \pm 15.36^{a}$ | $94.00 \pm 10.89^{a}$ |
| Serum glucose (mg/dl) | $69.04 \pm 8.56^{a}$ | $134.06 \pm 9.39^{b}$ | $96.25 \pm 6.19^{c}$ | $104.68 \pm 8.05^{c}$ | $107.71 \pm 3.48^{c}$ |
| Liver cholesterol (mg/g of wet tissue) | $3.23 \pm 0.34^{a}$ | $6.10 \pm 0.69^{b}$ | $3.95 \pm 0.90^{a}$ | $3.25 \pm 0.28^{a}$ | $5.00 \pm 0.22^{c}$ |
| Liver triglycerides (mg/g of wet tissue) | $5.27 \pm 0.43^{a}$ | $10.16 \pm 0.50^{b}$ | $8.93 \pm 0.30^{c,d}$ | $8.30 \pm 0.28^{d}$ | $9.28 \pm 0.31^{c}$ |
| Fecal cholesterol (mg/g of dry weight) | $2.26 \pm 0.41^{a}$ | $4.51 \pm 0.60^{b}$ | $7.03 \pm 0.68^{c}$ | $9.29 \pm 0.96^{d}$ | $5.03 \pm 0.68^{b}$ |

*Serum GPT*, serum glutamyl pyruvate transaminase; *Serum GOT*, serum glutamyl oxaloacetate transaminase; *NDC*, normal diet control; *HDC* hypercholesterolemic diet control; *HD-GG*, HD supplemented with *L. rhamnosus* GG; *HD-C38*, HD supplemented with *L. plantarum* VJC38; *HD-I21*, HD supplemented with *L. plantarum* VJI21. Results are expressed as means ± standard errors of the means; $n = 6$. Means in a row with different lowercase letters (a–e) are significantly different ($P < 0.05$)

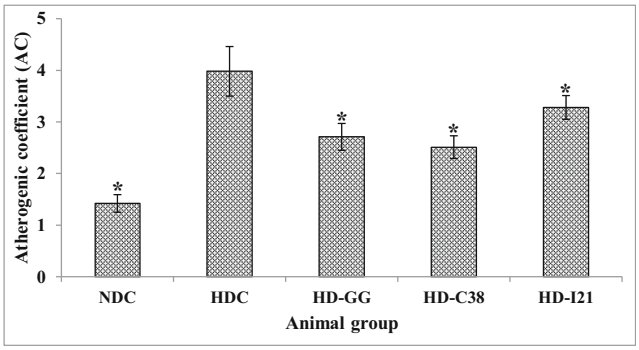


**Fig. 2** Atherogenic coefficient values of rats fed a normal diet (NDC), hypercholesterolaemic diet (HDC), or hypercholesterolaemic diet supplemented with *L. rhamnosus* GG (HD-GG) or *L. plantarum* VJC38 (HD-C38) or *L. plantarum* VJI21 (HD-I21). Data are expressed as means ± SEM ($n = 6$/group).* indicate significantly lower at $P < 0.05$ vs. the HDC group

developing CHD. Atherogenic coefficient value of probiotic test groups (HD-GG and HD-VJC38) decreased significantly when compared with hypercholesterolaemic diet control group. These results are in agreement with the earlier report (Kumar et al. 2011), where they showed significant decrease in atherogenic coefficient value of probiotic treated groups.

The BSH activity of LAB has often been linked with their hypocholesterolaemic activity (Kumar et al. 2011; Damodharan et al. 2015b). Ingestion of BSH active LAB might result in increased production of deconjugated bile acids in the small intestine where enterohepatic cycle takes place. Deconjugated bile acids are poorly absorbed from the small intestine due to their lower solubility under physiological conditions of small intestine (Wang et al. 2012). Thus, deconjugated bile acids are excreted more rapidly than conjugated bile acids. The increased excretion of bile acids could reduce quantities of bile acids available for reabsorption to liver. As a result, the feedback inhibition of bile salt synthesis decreased, and the synthesis of

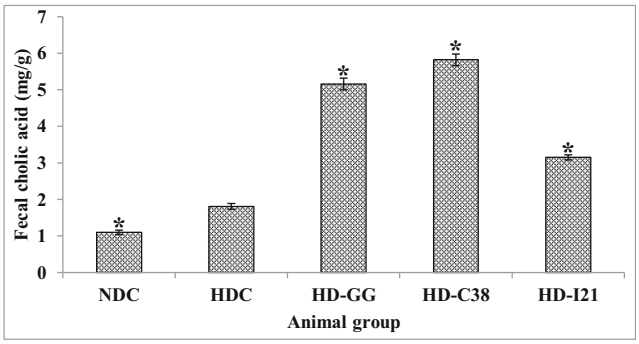


**Fig. 3** Fecal cholic acid levels of rats fed a normal diet (NDC), hypercholesterolaemic diet (HDC), or hypercholesterolaemic diet supplemented with *L. rhamnosus* GG (HD-GG) or *L. plantarum* VJC38 (HD-C38) or *L. plantarum* VJI21 (HD-I21). Data are expressed as means ± SEM ($n = 6$/group).* indicate significantly different at $P < 0.05$ vs. the HDC group

bile salts from cholesterol increased, thus providing the potential to reduce serum cholesterol levels (DeRodas et al. 1996). In this study, fecal cholic acid excretion was significantly high in the HD-C38 and HD-GG groups compared to other groups. In addition, the decrease in serum cholesterol by VJC38 and GG could be attributed to their ability to bind and assimilate cholesterol. The bound cholesterol by bacterial cells is less likely to be absorbed from the intestine to blood and excreted along with feces, thus providing potential to reduce body cholesterol. From these results, it can be hypothesized that strains VJC38 and GG might cause higher excretion of cholesterol and bile acids, thus resulting in decreased cholesterol in the serum and liver and increased excretion of cholesterol in the feces; this mechanism is similar to other studies (Taranto et al. 1998; Kumar et al. 2011; Wang et al. 2012). The present study indicated that *L. plantarum* VJC38 exhibited hypocholesterolaemic effect in rats fed hypercholesterolaemic diet through hydrolysis of conjugated bile acids in the small intestine and increased excretion of cholesterol in feces.

## Conclusion

*Lactobacillus plantarum* VJC38 exhibited significant reduction in serum T-CHO, LDL cholesterol, and TG level in rats, when compared with control HD group. Liver T-CHO and TG level were significantly decreased in HD group rats supplemented with *L. plantarum* VJC38, when compared with HD group rats without LAB. Fecal cholesterol and cholic acid levels were significantly higher in the HD group rats supplemented with *L. plantarum* VJC38 than other groups. These results indicated that *L. plantarum* VJC38 exhibited hypocholesterolaemic effect by hydrolysis of conjugated bile acids in the small intestine and enhanced fecal excretion of cholesterol. Hence, *L. plantarum* VJC38 may be used as a potential cholesterol-lowering probiotic. However, more clinical trials need to be conducted in adult humans with primary hypercholesterolemia to validate efficacy and safety this strain.

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## Compliance with ethical standards

**Conflicts of interest** The authors declare that they have no conflict of interest.

**Research involving human participants and/or animals** All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Before conducting the animal experiments, prior approval of the Institute's Animal Ethics Committee, Pondicherry

University was obtained and animal studies were performed in accordance with the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.



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