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Probiotic *Lactobacillus rhamnosus* GG and *Aloe vera* gel improve lipid profiles in hypercholesterolemic rats

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ABSTRACT

Objective: The effects of *Lactobacillus rhamnosus* GG (LGG) and *Aloe vera* (AV) gel on lipid profiles in rats with induced hypercholesterolemia were studied.
Methods: Five treatment groups of rats ($n = 7$) were the fed experimental diets: a normal control diet, a hypercholesterolemic diet (HD), HD + LGG, HD + AV gel, and HD + LGG + AV gel.
Results: Supplementation with LGG decreased serum total cholesterol by 32%; however, in combination with AV, the decrease was 43%. The decreases in triacylglycerol levels in the HD + LGG, HD + AV, and HD + LGG + AV groups were 41%, 23% and 45%, respectively. High-density lipoprotein increased by 12% in the HD + LGG + AV group, whereas very low-density and low-density lipoprotein values decreased by 45% and 30%, respectively. The atherogenic index in the HD + LGG + AV group decreased to 2.45 from 4.77 in the HD + LGG group. Furthermore, fecal *Lactobacillus* species counts increased significantly when LGG was fed in combination with the AV gel. The oral administration of LGG fermented milk alone or in combination with the AV gel increased cholesterol synthesis (3-hydroxy-3-methylglutaryl coenzyme A reductase expression) and absorption (low-density lipoprotein receptor expression), whereas cholesterol 7α-hydroxylase mRNA expression levels were lower in the HD + LGG and HD + LGG + AV groups compared with the control HD group.
Conclusion: The combination of LGG and AV gel may have a therapeutic potential to decrease cholesterol levels and the risk of cardiovascular diseases.

Introduction

Cardiovascular disease (CVD) is a major cause of death in adults in the Western world. Increased levels of certain blood lipids have been reported to be the principal cause of CVD and coronary heart disease [1]. Current dietary strategies for the prevention of CVD advocate an adherence to low-fat/low-saturated fat diets [2]. Although a low-fat diet may be an effective strategy to lower blood cholesterol concentrations in populations, the strategy appears to be less effective in individuals, largely due to poor compliance, attributed to low palatability and acceptability as a routine diet [3]. Consequently, attempts are being made to develop alternative dietary ingredients that can manage blood cholesterol levels. Supplementation of the diet with fermented dairy products or foods containing bifidobacteria and lactic acid bacteria have been reported to lower serum cholesterol levels [4–7].

Probiotics confer miscellaneous health benefits including an improvement in lactose intolerance, an increase in natural resistance to infectious disease in the gastrointestinal tract, the

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suppression of cancer, as an antidiabetic agent, and a decrease in serum cholesterol levels [5,6,8–10]. There have been reputable studies on the ability of probiotics to lower cholesterol levels in the body [11–13]. The possible mechanisms involved in the hypocholesteremic effect include an assimilation of cholesterol by growing cells, the binding of cholesterol to the microbial cellular surface, the deconjugation of bile by bile salt hydrolase (Bsh), the coprecipitation of cholesterol with deconjugate bile, the binding action of bile by fiber, and the microbial production of short-chain fatty acids (SCFAs) in the colon [7,14,15].

Aloe vera(AV) is a short-stemmed, succulentxerophyte that grows to 60 to 100 cm (24–39 inches) tall and spreads by offset shoots [16]. The phytometabolites in the plant have been found to be effective in the treatment of wounds, improving blood glucose levels in diabetics [17], lowering blood lipidsin hyperlipidemic patients [18], acute hepatic diseases [19], and decreasing symptoms and inflammation in patients with ulcerative colitis [20]. In addition, the AV extracts have antibacterial and antifungal activities that may help in the treatment of minor skin infections, such as boilsand benign skin cysts, and antifungal effects [21]. When used internally, AV gel improves the quality of the blood and helps rebalance the blood chemistry in a way that lowers cholesterol and total triacylglycerol (TAG) levels (in people with increased levels) [18,22,23]. The present study was undertaken to explore the combined therapeutic potential of probiotic *Lactobacillus rhamnosus* GG (LGG) and AV gel, with a particular focus on the cholesterol-lowering potential for lowering the risks of CVD.

Materials and methods

Experimental animals

Thirty-five male Wistar rats (each 110 d old) were allocated to one of five groups ($n = 7$/group). The animals were maintained in a central, small animal house at a controlled temperature of 22°C to 25°C and relative humidity of 56% to 60% and were fed a standard diet (AIN-76) and offered water ad libitum. Prior approval for study protocol was obtained from the animal ethics committee of the institute registered under the National Committee for the Purpose of the Control and Supervision of Experiment on Animals, Government of India.

Preparation of fermented milk and its feeding

Skim milk containing not more than 0.5% milk fat and 9.7% non-fat solids was used for the maintenance of the bacterial culture and preparation of probiotic-fermented milk. Because LGG (ATCC 53103) normally requires additional sugars, 2% (w/v) D-mannitol was used to fortify the milk followed by sterilization by momentary pressure. The probiotic LGG ($\sim 10^8$ cfu/mL) was used as an inoculum for preparing the fermented milk. The inoculated milk was incubated for 15 to 18 h at 37°C.

Preparation of AV gel

The AV leaves were freshly harvested by cutting from the bottom and were washed thoroughly. The edges were removed and the leaves were peeled to obtain the slimy and transparent gel contents. The gel that was scooped out from a single plant was transferred to a sterilized amber-colored glass bottle with an air-tight lid and was preserved in the refrigerator for further experimentation. The gel extract was orally administered at a dose of 500 mg/kg of body weight per day per animal in the respective groups.

Diet and experimental design

The base composition of the experimental diet is presented in Table 1. The animal diets were formulated based on the AIN-76 formulation. The five experimental diets included a normal control diet, a high-cholesterol diet (HD), an HD with LGG (HD + LGG), an HD with AV gel (HD + AV), and an HD with LGG and AV gel (HD + LGG + AV).

Blood sampling and analytical procedures

The overnight-fasted rats were bled from the orbital venous plexus at day 45 for plasma lipid analysis. The blood was collected in heparinized sterile microfuge tubes and centrifuged at 2000 × *g* for 15 min at 4°C. The samples were analyzed for total cholesterol, TAG, and high-density lipoprotein (HDL) cholesterol using commercial enzymatic kits (Autopak, M/s Siemens Diagnostics Ltd., Gujarat, India). The Friedewald equation [24] was applied to analyze the following, other plasma lipid fractions: 1) low-density lipoprotein (LDL) cholesterol = total cholesterol−HDL cholesterol−(TAG/5), with all concentrations as milligrams per liter; 2) very low-density lipoprotein (VLDL) cholesterol = (TAG/5), with this quotient used as an estimate of VLDL cholesterol; and 3) atherogenic index (AI) = (total cholesterol−HDL cholesterol)/HDL cholesterol.

Table 1
Composition of high-cholesterol diet

Constituents	g/100 g
Starch	19.0
Casein	20.0
Hydrogenated vegetable oil	10.0
Refined oil (soybean)	10.0
Vitamin mixture*	1.0
Mineral mixture†	4.0
Choline chloride	0.2
Methionine	0.3
Cholesterol	0.5
Sucrose	30.0
Cellulose powder	5.0

The normal diet was defined as the high-cholesterol diet minus the cholesterol (cellulose was added for compensation)
* AIN-76 vitamin mixture.
† AIN-76 mineral mixture.

Analysis of expression of genes related to lipid metabolism

Rats were euthanized by cervical separation and liver samples were collected for mRNA quantification. The middle lobe of each rat liver was used as the sampling region, based on previous studies on the gradient distribution of cholesterol 7α-hydroxylase expression (*CYP7A1* gene) in the rat liver [25]. Dissected liver samples were rinsed with diethylpyrocarbonate-treated phosphate buffered saline (0.1% diethylpyrocarbonate in 0.01 M phosphate buffered saline, pH 7.4) and stored in RNALater (Sigma, St. Louis, MO, USA) at −20°C. Relative expression levels of the mRNA for different genes were evaluated by reverse transcriptase polymerase chain reaction (PCR). Total RNA was extracted using TRI reagent (Sigma) and cDNA was synthesized from 1 μg of total RNA using the RevertAid first-strand cDNA synthesis kit (Fermentas, UAB, Lithunia). The cDNAs were analyzed for mRNA expression levels; PCR analysis was carried out using 2.0 μL of cDNA, 25 μL of the PCR master mix (Fermentas), and 1.0 μL of primer (10 pmol each) upstream and downstream (Table 2). The initial PCR cycle consisted of denaturation at 94°C for 5 min, annealing at 58°C for 40 s, and extension at 72°C for 40 s, followed by 29 cycles at 94°C for 30 s, 58°C for 40 s, and extension at 72°C for 40 s, with a terminal extension at 72°C for 5 min. The

Table 2
List of primer sequence for reverse transcriptase polymerase chain reaction and the amplicons obtained for different genes

Gene	Primers (5′–3′)	Amplicon size (bp)	Reference
LDL receptor		532	present study
Upstream primer	5′-GGGCGTGCAGCTCCCCAC-3′		
Downstream primer	5′-TCCGCCCGGGACTGTCGT-3′		
HMG-CoA reductase		361	present study
Upstream primer	5′-CGCTGGCAGGACGCAACC-3′		
Downstream primer	5′-CGATGTTGGCAGCATGGG-3′		
Cholesterol 7á-hydroxylase		650	present study
Upstream primer	5′-ATCTTGGCATGGCCCTGA-3′		
Downstream primer	5′-GAGCATCTCCTGCCTCTC-3′		
GAPDH		942	present study
Upstream primer	5′-CGTATCGGACGCCTCGTT-3′		
Downstream primer	5′-GTCCACCACCCTGTTGCT-3′		

GAPDH, glyceraldehyde 3-phospate dehydrogenase; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; LDL, low-density lipoprotein

Table 3
Serum TC, TAG, HDL, LDL, and VLDL cholesterol concentrations in Wistar rats fed the experimental diets

Lipid profile	NDC	HDC	HD + LGG	HD + AV	HD + LGG + AV
Serum TC (mg/dL)	145.28 ± 1.92^{a}	348.85 ± 2.61^{b}	234.71 ± 6.27^{c}	252.28 ± 4.41^{d}	197.71 ± 3.26^{e}
Serum TAG (mg/dL)	72.28 ± 2.10^{a}	118.14 ± 3.57^{b}	73.42 ± 3.43^{a}	85.85 ± 2.95^{c}	64.42 ± 2.9^{d}
HDL (mg/dL)	64.85 ± 2.20^{a}	52.14 ± 4.07^{b}	41.71 ± 2.47^{c}	44.85 ± 3.38^{c}	58.71 ± 2.88^{b}
VLDL (mg/dL)	15.54 ± 0.87^{a}	23.02 ± 3.74^{b}	13.66 ± 0.13^{c}	17.69 ± 0.24^{d}	12.48 ± 0.14^{e}
LDL (mg/dL)	49.38 ± 2.49^{a}	145.45 ± 3.48^{b}	71.41 ± 4.24^{c}	92.68 ± 1.98^{d}	101.73 ± 2.34^{e}
LDL/HDL ratio	0.74 ± 0.012^{a}	2.86 ± 0.19^{b}	1.74 ± 0.17^{c}	2.12 ± 0.15^{d}	1.75 ± 0.098^{c}
TC/HDL ratio	2.24 ± 0.055^{a}	6.92 ± 0.51^{b}	5.76 ± 0.43^{c}	5.84 ± 0.49^{c}	3.40 ± 0.16^{d}
AI*	1.24 ± 0.05^{a}	5.92 ± 0.51^{b}	4.77 ± 0.43^{c}	4.82 ± 0.48^{c}	2.45 ± 0.16^{d}

AI, atherogenic index; HD + AV, high-cholesterol diet containing *Aloe vera*; HD + LGG, high-cholesterol diet containing *Lactobacillus rhamnosus* GG; HD + LGG + AV, high-cholesterol diet containing *Lactobacillus rhamnosus* GG and *Aloe vera*; HDC, control high-cholesterol diet; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NDC, control normal diet; TAG, triacylglycerol; TC, total cholesterol; VLDL, very low-density lipoprotein
Results are expressed as mean ± SEM ($n = 6$). Means within a row with different superscript letters are significantly different ($P \leq 0.05$).
* AI = (TC−HDL cholesterol)/HDL cholesterol.

PCR products were electrophoresed in 1.5% agarose gels. LabWorks 4.0 (UVP, Inc., Upland, CA, USA) was used for the gel image acquisition, band identification, and determination of band optical density. The band optical density of glyceraldehyde 3-phospate dehydrogenase (GAPDH) was used as the baseline, and the relative densities of the LDL receptor, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, and cholesterol 7α-hydroxylase were compared with that of GAPDH.

Microbiological analysis of fecal samples for Lactobacillus species counts

Fecal samples from the experimental animals were collected after a 15-d interval in separate sterile tubes for microbial analyses and were processed within 1 h of collection. Each sample was homogenized with a stomacher using sterile phosphate buffered saline and peptone saline diluents. Subsequent 10-fold serial dilutions of each sample were plated in triplicate on MRS agar (Hi-Media Pvt. Ltd., Mumbai, India) and incubated anaerobically at 37°C for 48 h.

Oral glucose tolerance test

At day 45 after the dietary treatment, the rats were deprived of the diets for 12 h and then given glucose solution (5 g/kg) by intragastric gavage. Blood samples were drawn from the tail for oral glucose tolerance testing. Serum glucose was measured at 0, 30, 60, 90, and 120 min by a fast blood glucose meter.

Statistical analysis

Data analysis was carried out with SPSS 10.0 (SPSS, Inc., Chicago, IL, USA). One-way analysis of variance was used to determine significant differences between means, with a significance level of $P \leq 0.05$. Critical difference values were used to perform multiple comparisons between means. All data are presented as mean ± standard error of the mean ($n = 6$).

Results

The effects of the dietary treatments on the serum lipid profile (plasma total cholesterol, HDL cholesterol, LDL cholesterol, VLDL cholesterol, TAG, and AI) are presented in Table 3. Groups HD + LGG, HD + AV, and HD + LGG + AV showed 32%, 27%, and 43% decreases in serum total cholesterol, respectively, compared with the HD group after 45 d of dietary treatment. These three groups also exhibited a decrease in LDL cholesterol at a significant level. The decreases in LDL cholesterol were 50%, 36%, and 30% in the HD + LGG, HD + AV, and HD + LGG + AV groups, respectively, whereas HDL cholesterol showed a similar but opposite trend. HDL cholesterol values for the HD + LGG + AV group were increased by 12%, whereas decreases of 20% and 13% were observed in groups HD + LGG and HD + AV, respectively.

The statistical analysis showed that plasma HDL cholesterol concentrations differed significantly among the experimental groups ($P < 0.05$). Similarly, plasma TAG and VLDL cholesterol concentrations differed significantly ($P < 0.05$) among all the groups throughout the experiment. After the dietary treatments, TAG and VLDL cholesterol levels in the HD + LGG, HD + AV, and HD + LGG + AV groups decreased by 41%, 23%, and 45%, respectively. The AI values of the experimental groups also differed significantly ($P < 0.05$). The AI values of the treatment groups increased sharply after feeding with the HD compared with that recorded with the control diet group. However, the maximum decrease was found for the AI of the HD + LGG + AV group after 45 d of treatment compared with the HD group.

The finding that combinational feeding with LGG and AV gel had the most effective antihypercholesterolemic effect followed by feeding with LGG alone was well supported by the results of the fecal *Lactobacillus* species counts taken every 15 d during the study period (Table 4). There was a significant increase in the counts from 8.95 log cfu on first day to 9.94 log cfu on the 45th day in the HD + LGG + AV group, whereas in the group where LGG was fed without AV gel, the counts increased to 9.71 log cfu, which was significantly lower than in the former group. The results indicate that AV gel had a positive effect on the growth of lactobacilli.

The feeding of LGG and AV gel together or separately also had a significant controlling effect on the weight gain in the HD group (Table 5). The weight gains during the study period were 58.77 g with the control diet and 88.85 g with the HD diet. However, when feeding the LGG and AV gel separately but with HD diet, the weight gain was significantly less, i.e., 75.20 and 79.63 g, respectively, compared with the HD group, clearly supporting the antihypercholesterolemic attributes of the probiotic LGG and the AV gel. Furthermore, in group HD + LGG + AV, the weight gain was maintained at 68.61 g, which was significantly less than that observed in groups fed with LGG and AV gel separately.

Table 4
Lactobacillus species counts in rat feces at days 0, 15, 30, and 45 of feeding

Group	Log_{10} cfu			
	0 d	15 d	30 d	45 d
NDC	8.84 ± 0.04^{ap}	8.94 ± 0.04^{bp}	9.01 ± 0.02^{cp}	9.10 ± 0.08^{cp}
HDC	8.89 ± 0.05^{aq}	8.93 ± 0.04^{ap}	8.86 ± 0.05^{aq}	8.98 ± 0.03^{ap}
HD + LGG	8.95 ± 0.09^{aq}	9.31 ± 0.05^{bq}	9.62 ± 0.09^{cr}	9.71 ± 0.08^{cq}
HD + AV	8.91 ± 0.04^{aq}	8.87 ± 0.06^{ap}	8.97 ± 0.08^{aq}	9.06 ± 0.06^{ap}
HD + LGG + AV	8.95 ± 0.07^{aq}	9.62 ± 0.07^{br}	9.81 ± 0.06^{cs}	9.94 ± 0.06^{dr}

HD + AV, high-cholesterol diet containing *Aloe vera*; HD + LGG, high-cholesterol diet containing *Lactobacillus rhamnosus* GG; HD + LGG + AV, high-cholesterol diet containing *Lactobacillus rhamnosus* GG and *Aloe vera*; HDC, control high-cholesterol diet; NDC, control normal diet
Results are expressed as mean ± SEM ($n = 6$). $^{a–d}$ Mean values within a row with different superscript letters differ significantly ($P < 0.05$); $^{p–s}$ mean values within a column with different superscript letters differ significantly ($P < 0.05$).

Table 5
Initial weight, final weight, weight gain, and feed intake in rats

	NDC	HDC	HD + LGG	HD + AV	HD + LGG + AV
Initial weight (g)	161.16 ± 1.88	167.49 ± 2.45	165.51 ± 2.22	165.71 ± 2.27	166.07 ± 1.99
Final weight (g)	219.94 ± 2.48	256.30 ± 3.99	240.71 ± 3.26	245.35 ± 2.90	234.68 ± 3.46
Weight gain (g)	58.77 ± 2.90[a]	88.85 ± 1.81[b]	75.20 ± 2.88[c]	79.63 ± 4.30[c]	68.61 ± 3.23[d]
Feed intake (g)	141.84 ± 1.96[a]	161.5 ± 1.68[b]	158.80 ± 2.61[b]	169.12 ± 2.44[c]	160.86 ± 0.98[b]

HD + AV, high-cholesterol diet containing *Aloe vera*; HD + LGG, high-cholesterol diet containing *Lactobacillus rhamnosus* GG; HD + LGG + AV, high-cholesterol diet containing *Lactobacillus rhamnosus* GG and *Aloe vera*; HDC, control high-cholesterol diet; NDC, control normal diet
Results are expressed as mean ± SEM ($n = 6$). Mean values within a row with different superscript letters differ significantly ($P < 0.05$).

Hepatic mRNA expression levels for cholesterol metabolism-related genes, i.e., HMG-CoA reductase, LDL receptor, cholesterol 7α-hydroxylase, and GAPDH, were determined by specifically primed (Table 2) PCR amplifications that resulted in different product sizes (Table 2 and Fig. 1). Relative mRNA levels (percentages) for each target gene differed significantly ($P \leq 0.05$) among the dietary treatment groups (Fig. 1). The inclusion of dietary cholesterol resulted in a significant ($P \leq 0.05$) downregulation of *hmgr* mRNA expression in the HD control group, whereas oral supplementation of LGG and LGG + AV upregulated the respective mRNA expressions compared with the HD control group (Fig. 1). A similar mRNA expression pattern was recorded for the cholesterol 7á-hydroxylase gene, whereas LDL receptor mRNA levels were higher in the treatment groups, namely HD + LGG, HD + AV, and HD + LGG + AV, compared with the control HD and normal diet groups.

Discussion

It is well known that increased cholesterol levels constitute the predisposing factor associated with an increased risk of CVD. Hence, lowering serum/plasma cholesterol (total and LDL) in hypercholesterolemic patients lowers the incidence of CVD. A probiotic dietary intervention could be a promising and cost–effective approach in lowering serum/plasma cholesterol levels and, hence, may play a crucial role in the management of CVD. Probiotic lactobacilli are considered normal components of the intestinal microflora in humans and animals and have been associated with various health-promoting properties. LGG, a proved probiotic strain, has rarely been tested for its cholesterol-lowering potential. Hence, this study was designed specifically to test the antihypercholesterolemic potential of LGG fermented milk alone or in combination with AV gel in a rat model. The present results clearly showed that LGG fermented milk alone and in combination with AV gel lowered serum total cholesterol, LDL cholesterol, and TAG levels in groups HD + LGG and HD + LGG + AV. The oral administration of LGG fermented milk in combination with AV gel resulted in maximum decreases of serum total cholesterol and TAG by 43% and 45%, whereas LDL cholesterol levels were decreased by 50% in the HD + LGG group. These observations are consistent with previously published studies on the antihypercholesterolemic effect of probiotic-fermented foods [26,27]. In one such study, the effect of *Lactobacillus acidophilus* and dietary yogurt on mice plasma lipids and TAG levels was examined, and significant decreases of 17% and 33% were observed in total and LDL cholesterol levels, respectively, in the *L. acidophilus* group. However, decreases of total and LDL cholesterol by 7% and 11% in the ordinary-yogurt group were not significant ($P > 0.05$), which suggested that *L. acidophilus* can colonize efficiently in the mouse gastrointestinal tract [26]. In a similar study, Xiao et al. [27] reported the lowering of serum concentrations of total cholesterol, LDL cholesterol, and TAG in rats fed a diet supplemented with lyophilized powder of fermented milk with yogurt starters and *Bifidobacterium*, whereas no change in HDL cholesterol concentration was observed. Furthermore HDL cholesterol levels were partly increased in rats fed with LGG and AV, which shows that the oral administration of LGG fermented milk alone or in combination with AV gel did not affect serum HDL cholesterol concentrations. This is perhaps the first study in which the antihypercholesterolemic potential of LGG fermented milk plus AV gel has been demonstrated in Wistar rats fed a hypercholesterolemic diet.

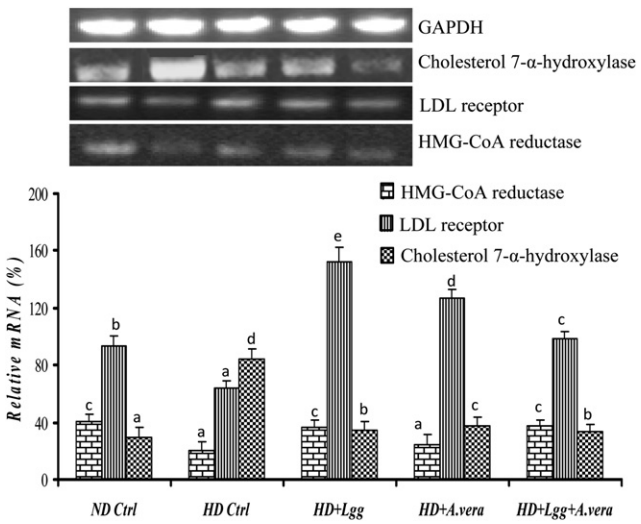

Fig. 1. Hepatic mRNA expression levels for the HMG-CoA reductase, LDL receptor, and cholesterol 7α-hydroxylase genes. Results are expressed as mean ± SEM ($n = 6$). Mean values within treatment groups with different lowercase letters are significantly different ($P \leq 0.05$). GAPDH, glyceraldehyde 3-phospate dehydrogenase; HD + *A. vera*, high-cholesterol diet + *Aloe vera*; HD + Lgg, high-cholesterol diet + *Lactobacillus rhamnosus* GG; HD + Lgg + *A. vera*, high-cholesterol diet + *Lactobacillus rhamnosus* GG + *Aloe vera*; HD Ctrl, control high-cholesterol diet; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; LDL, low-density lipoprotein; ND Ctrl, control normal diet.

The cholesterol levels in blood are regulated at different levels, namely absorption, synthesis, and excretion. However, LDL receptor–mediated endocytosis controls plasma cholesterol levels by hepatic absorption, and the LDL receptor is regulated by a transcriptional control mechanism [28]. A regulatory enzyme, HMG-CoA reductase, in the cholesterol synthesis pathway catalyzes the synthesis of mevalonate from HMG-CoA [29] and is regulated at the post-transcriptional level.

Fecal bile excretion is the only direct path for decreasing the level of cholesterol, and hepatic cholesterol 7α-hydroxylase regulates the bile acid synthesis from cholesterol. Cholesterol synthesis (HMG-CoA reductase) and absorption (LDL receptor) represent the cholesterol inputs to the liver, although bile acid synthesis (cholesterol 7α-hydroxylase) is a measurement of a decrease in hepatic cholesterol. A hypercholesterolemic diet

Table 6
Effect of *Lactobacillus rhamnosus* and *Aloe vera* on serum glucose (millimoles per liter)

	NDC	HDC	HD + LGG	HD + AV	HD + LGG + AV
0 min	4.62 ± 0.15^{ap}	5.15 ± 0.10^{bp}	5.11 ± 0.10^{bp}	5.12 ± 0.12^{bp}	4.82 ± 0.18^{ap}
30 min	7.50 ± 0.10^{aq}	7.68 ± 0.11^{aq}	7.57 ± 0.08^{aq}	7.62 ± 0.12^{aq}	7.42 ± 0.12^{aq}
60 min	6.87 ± 0.09^{ar}	7.51 ± 0.16^{bq}	7.49 ± 0.03^{bq}	7.52 ± 0.06^{bq}	7.31 ± 0.12^{cq}
90 min	6.14 ± 0.07^{as}	7.31 ± 0.12^{bq}	6.68 ± 0.05^{cr}	6.77 ± 0.08^{cr}	6.34 ± 0.17^{ar}
120 min	4.94 ± 0.08^{at}	7.04 ± 0.13^{br}	6.25 ± 0.23^{cs}	6.47 ± 0.29^{cr}	5.61 ± 0.23^{ds}

HD + AV, high-cholesterol diet containing *Aloe vera*; HD + LGG, high-cholesterol diet containing *Lactobacillus rhamnosus* GG; HD + LGG + AV, high-cholesterol diet containing *Lactobacillus rhamnosus* GG and *Aloe vera*; HDC, control high-cholesterol diet; NDC, control normal diet
Results are expressed as mean ± SEM ($n = 6$). [a–d] Mean values within a row with different superscript letters differ significantly ($P < 0.05$); [p–t] mean values within a column with different superscript letters differ significantly ($P < 0.05$).

decreases the input of cholesterol to the liver, especially that from cholesterol synthesis (HMG-CoA reductase). Bile acids, cholesterol, and mevalonate inhibit HMG-CoA reductase expression [28], and the present study demonstrated that supplementation of the normal diets with cholesterol had an inhibitory effect on the HMG-CoA reductase expression. Supplementation with LGG and/or AV augmented the hepatic HMG-CoA reductase mRNA expression compared with the control HD group ($P < 0.05$). Cholesterol absorption through the LDL receptor (LDL receptor mRNA expression) was not found to be affected by dietary cholesterol supplementation; however, LDL receptor mRNA expression was upregulated in the group orally supplemented with LGG and AV ($P < 0.05$). The reaction by cholesterol 7α-hydroxylase is the rate-limiting step in bile acid synthesis from cholesterol, and its transcription and activity are increased by endogenous and dietary cholesterols [30]. Dietary cholesterol supplementation resulted in increased bile acid synthesis (percentage of increase in cholesterol 7α-hydroxylase mRNA expression) and supplementation with LGG plus AV gel decreased the hepatic cholesterol 7α- hydroxylase mRNA expression ($P < 0.05$).

The probiotic bacteria ingested through fermented foods lower lipid levels through different mechanisms. Bile acid amino (glycine/taurine) conjugates pass through the small intestine and are hydrolyzed by Bsh active lactobacilli and bifidobacteria. Free bile acids formed by the deconjugation of conjugated bile salts are less soluble, are less likely to be reabsorbed by the intestinal lumen compared with their conjugated counterparts, and are lost through the feces [31], thus leading to a decrease of serum cholesterol [32].

This study indicated that AV gel plus LGG exhibit hypocholesterolemic effects (Table 2). It is evident that the microbiome in the large intestine is active in fermenting dietary carbohydrates and other unabsorbed polysaccharides to produce various metabolites, including SCFAs [33,34]. The production of cholesterol appears to be affected by the relative amounts of volatile fatty acids produced in the gastrointestinal tract [35]. However, the SCFA production could not be measured in this study because the large intestine readily absorbs SCFAs while they are synthesized and metabolized [36]. To find out how much of the acetate produced in the colon actually ends up in the blood circulation, Wolever et al. [37] studied the interaction between SCFAs in the colon and in blood serum and found that sodium acetate or sodium propionate in the rectum could increase their levels in the blood. It was inferred that acetate causes an increase in total cholesterol and a decrease in fatty acids, whereas propionate increases blood glucose and decreases the cholesterol-lowering response stimulated by acetate [37].

The peak of serum glucose in the control groups appeared at 30 min, returned to normal at 120 min, and was found to be significantly lower ($P < 0.05$) in control diet group (4.94 U) than that in HD group (7.04 U) in the present study (Table 6). In the HD groups, the peak was postponed to 60 to 120 min and lasted longer than in the control groups. In these groups, serum glucose did not return to normal until 120 min (Table 6), and the results obtained were consistent with the finding of Yadav et al. [38] who too observed similar pattern for serum glucose levels for rats fed on probiotic dahi containing *L. acidophilus* and *Lactobacillus casei*.

There has been a surge of interest in using phytometabolites for nutritional and health applications [39]. The present study showed that probiotic-fermented milk alone or in combination with AV gel had a positive effect on the lipid profile in experimental animals, although the mechanisms involved warrant further investigations. Nassiff et al. [40] found that the oral administration of AV gel extract (300 mg/kg of body weight per day) to diabetic rats for 21 d resulted in significant decreases in plasma and tissue (liver and kidney) cholesterol, TAG, free fatty acid, and phospholipid profiles. In addition, the decreased plasma levels of HDL cholesterol and increased plasma levels of LDL and VLDL cholesterol in diabetic rats were restored to near normal levels after treatment with the extract [40]. Other studies have reported that AV gel can lower hepatic TAG levels and VLDL production rates [40,41]. Furthermore, AV gel was found to promote the growth of probiotic lactobacilli as evident from the increased fecal *Lactobacillus* species counts (Table 4), which could be attributed to growth-promoting components such as mannose, glucose, L-rhamnose, vitamins, amino acids, and trace elements present in AV [40–42]. This indicates that, in addition to lowering or controlling cholesterol levels, AV gel promoted the growth of LGG, which further lowered the cholesterol levels by the assimilation, deconjugation, or metabolism of bile salts and led to the efficient management of the lipid profiles and AI.

In conclusion, the present study has presented the antihypercholesterolemic effect of the probiotic LGG plus AV gel in rats with induced hypercholesterolemia, where serum cholesterol, LDL, VLDL, and TAG levels were found to be decreased significantly, and HDL cholesterol levels were observed to be increased significantly. Therefore, an optimized blend of the probiotic LGG and AV gel could be exploited as a potential biotherapeutic remedy to decrease cholesterol levels and lower the risk of CVD, although the field is open for further studies.

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