Effect of Probiotic Bifidobacterium Bifidum PTCC1644 on Lipid Profile in Rat

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Abstract: Background: The preventive effects of probiotics on hyperlipidemia have been reported in recent studies. Bifido bacteria as type of lactic acid bacteria, among probiotics, are one of the most numerous probiotics in the mammalian gut. The present aims to investigate the effect of Bifidobacterium bifidum PTCC1644 on lipid profile in rat serum. **Methods:** The sample of eighteen male rat of Wistare race were assigned into three groups as follows: SD group, fed standard diet; FSD group, fed fat diet; FSD-LAB group, fed fat diet supplemented with LAB (Bifidobacterium strain). At the first (8 week) and end (17 week) of study, lipid serum levels (TC, HDL-C, LDL-C, TG) were measured. **Results:** B. bifidum PTCC1644 decreased serum total cholesterol, LDL- cholesterol and triglycerides; it also increased HDL- cholesterol slightly in FSD-LAB than FSD groups. **Conclusion:** the results showed that daily consumption of Bifidobacterium strain used in this study can be effective in managing serum lipid profile and coronary heart disease.
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Introduction:

Cardiovascular diseases (CVDs) are considered as the major reason of death globally, many people die due to CVDs than other causes [1]. CVDs associated with hyperlipidemia are leading causes of death in Iran, like many other countries. Studies done on Animal and human have shown that the reduction of cholesterol or low-density-lipoprotein (LDL) in plasma may lead to reduction of risk associated with CVDs [2]. Living microbial supplement is referred to Probiotics that improve host animal's intestinal microbial balance [3]. A number of therapeutic role have been reported for probiotics like lowering plasma lipids [4, 5]. Bifidobacteria as a type of lactic acid bacteria are the major bacteria in the intestines of newborn babies and one of the most numerous probiotics in the gut. These bacteria have been used extensively and well-tolerated.

In one study, a Subspecies of Bifidobacterium longum revealed a significant effect in lowering Serum total cholesterol than a mixed culture of Streptococcus thermophilus and Lactobacillus delbrueckii Subspecies bulgaricus both in rat and human [6]. In another study, Bifidobacterium longum SPM1207 lowered serum total cholesterol and LDL levels significantly, and slightly increased serum HDL[7]. Another randomized, placebo-controlled and parallel designed study was done to check the ability Lactobacillus plantarum CK102 in cholesterol lowering effect. In this study, thirty-two Sprague-Dewls(SD) male rats fed with $500 \times 10^7 (\frac{cfu}{ml})$ daily to 6 week and the result showed that concentration of total cholesterol compared to the control was reduced significantly by 50.3% [8]. The present study aimed to investigate the effect of B. bifidum PTCC1644 on lipid profile in rat serum.

Material and methods

Bacterial strain and culture

Table 1 shows the origins of the strain and culture media and methods used in this study. B. bifidum PTCC (Persian Type Culture Collection-Iran) 1644 was provided by microbial collection from the department of food and drug control, faculty of pharmacy, Tehran university medical sciences. The selected B. bifidum PTCC1644 was resistant to acidic PH[9].

Table 1 Culthure media and procedure

Media	Main counted microorganisms	Incubation time(day)
Anaerobic cultur[a]		
MRS agar and broth medium[b]	***Bifidobacterium bifidum* PTCC1644**	**2**

[a] → Culture media were prepared and dispensed into plates on a clean bench under the laboratory biologic hood. plates were incubated in a CO_2 incubator (Memert. Germany) with anaerobic jars ($H_2/CO_2/N_2$; 5:5:90 WhitleyJar.UK). A sample of each dilution was plated on each medium under the laboratory biologic hood. Pelates were incubated in anaerobic condition at 37°C for 48- h.

[b] → MRS, deMan Rogosa Sharp (Merck, Germany)

B. bifidum was inoculated in 200 ml deMan-Rogosa-Sharp (MRS) broth, the cultured media incubated at 37°C in anaerobic conditions for 48-h, bacterial suspension were diluted serially 10 fold with MRS broth and turned in to 10^{-1} to 10^{-8} into tubes ($1\frac{ml}{tube}$). The tubes were stored at 4°C to be used later. The content of each tube with concentration $10^8\left(\frac{cfu}{ml}\right)$ was used as a unit of bacterial daily dose through oral gavage to each rat. The bacterial viability and its concentration were checked with the application of MRS agar plating during the storage period [10].

Animal and treatment

A total of 18 Wistare male rat (7-week-old) were purchased from Pasteur Institute (Iran) and were maintained in stainless steel cages with room temperature – controlled(22 ± 2°C) with a 12h light/dark cycle and humidity $55\pm5\%$.

All the animals were fed on a standard diet for 1week. After this adaptation period, the rats were assigned into three experimental groups randomly, each groups contains six rats according to the type of diet and test material. As shown in Table2, group1 (SD) was fed a standard diet and MRS broth ($1\frac{ml}{day}$); group2(FSD) was fed a 1% cholesterol mixed to standard diet and MRS broth($1\frac{ml}{day}$); and group3 (FSD-LAB) was fed a 1% mixed to standard diet and MRS broth containing Bifidobacterium bifidum $10^8 - 10^9\left(\frac{cfu}{ml}\right)$ once a day.

Sampling and Analytical Procedures

At the first (8-week-old) and end (17-week-old) of the study, fasting blood samples were collected by jugular vein under diethyl ether anaesthesia. The serum was separated from the blood by centrifugation at 4.000 rpm for 10 min. The total-cholesterol(TC), high density lipoprotein-cholesterol(HDL-C),low density lipoprotein-cholesterol(LDL-C) and triglyceride levels in the serum were determined by an enzymatic colorimetric methods[11-13], using commercially analytic kits (Pars azmoon. Iran) and kinetic UV assay using a auto-analyser (Hitachi 707- Germany and Japan) in Lab (Noor Pathobiology. Iran).

Statistical analysis

All the data are presented as Mean$\pm$Standard Deviation (SD). Two T-test was used (spss version 17) to compare data before and after intervention in each group. P value of less than 0.05 showed statistical significance.

Table 2. Feeding schedules used in the present study

Groups	Rat	Food	Treatment (oral administration for 9 week)
SD	**6**	**standard diet**	**sterilized MRS broth (1ml)**
FSD	**6**	**standard diet mixed 1% cholesterol**	**sterilized MRS broth (1ml)**
FSD-LAB	**6**	**standard diet mixed 1% cholesterol**	**sterilized MRS broth (1ml) containing LAB (10^8cfu)**

Results

Triglycerides

Changes Triglyceride concentration in different groups is shown in Figure 1.The end of study in compared with start, the amount of Triglycerides showed meaningful statistical change in FSD group($P<0.05$) but didn't show any statistical meaningful change in SD and FSD-LAB groups.

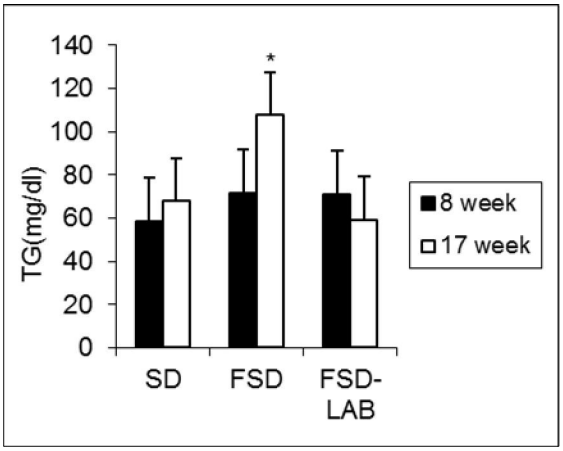

Figure 1: Effect of B. bifidum PTCC1644 on Triglyceride levels.

The entire animal was adapted to the standard diet for 1week. Then the rats in group 1 to 3 received daily 1 ml MRS broth or MRS broth containing B. bifidum PTCC1644($10^8 - 10^9 \frac{cfu}{ml}$)

*p < 0.05 significantly different compared with 8 week

Total cholesterol

Table 3 shows the changes in Total cholesterol concentration. The amount of TC serum rats in FSD group showed significant increment in the increment of TC after 9 weeks (17 weeks than 8 weeks) (P<0.05); but with spite of small reduction and increment of TC in SD and FSD-LAB groups, no meaningful statistical change was observed (P>0.05).

Low – density lipoprotein cholesterol

Table 3 shows the changes in LDL-C concentration. During the study, the amount of LDL-C showed meaningful statistical change in FSD and FSD-LAB groups (P<0.05); but in spite of small increment in LDL-C in SD group, no meaningful statistical change was observed (P>0.05).

High – density lipoprotein cholesterol

Table 3 shows the changes in HDL-C concentration. The amount of HDL-C serum rats in FSD group showed meaningful change in the reduction of HDL-C after 9 weeks (17 weeks than 8 weeks) (P<0.05);but in spite of small reduction of HDL-Cin FSD-LAB and SD groups, no meaningful statistical change was observed didn't show any statistical meaningful change in SD and FSD-LAB groups (P>0.05).

Discussion

Cardiovascular diseases are considered as the major reason of death globally [1]. Hypercholesterolemia is strongly associated with coronary heart disease and atherosclerosis [14-16]. The risk of heart attack is increased three times higher with hypercholesterolemia, compared to those who have normal blood lipid profile [17]. Probiotics as an alternative biological method for chemical agents in managing the reduction of serum lipid levels have attracted much attention. The potential hypocholesterolemic activity of bacterial strains such as Lactobacillus sp. and Bifidobacteium sp. have been the focus of many studies [18-21]. In our study the effect of B. bifidum PTCC1644 in reduction of rat's Triglyceride level in FSD-LAB was observed when compared with FSD group. On the other hand, a small increment was observed in the amount of TC level in FSD-LAB in comparison with the beginning of study (week 8). But according to the increment of TC in FSD-LAB and FSD groups which were 3.52 % and 42.6% respectively at the end of the study(week 17) , this finding shows that B. bifidum PTCC1644 might have a reduction effect on TC level. Investigating of HDL-C serum rats at the first(week 8) and end of (week 17) the study,although the reduction of HDL-C serum rats was observed in all groups, there was also lower reduction in FSD-LAB group in comparision with FSD (24.88% and 29.44% respectively), this finding shows that B. bifidum PTCC1644 might have an increment effect on HDL-C level. Investigating of LDL-C serum rats at the first (week 8) and end of (week 17) the study , showed an increment in the amount of LDL-C in all groups but there was also lower increment in FSD-LAB in comparison with FSD- groups (126/47% and 256.24% respectively), this finding shows that B. bifidum PTCC1644 might have a reduction effect on LDL-C level.

These results are in agreement with other studies.

Abeer et al results indicated that, supplementation with Bifidobacterium and Lactobacillus acidophilus alone and in combination significantly decreased the mean value of serum Total cholesterol, LDL-cholesterol and Triglycerides but increased HDL-cholesterol [19]. In other experiments, Hyang et al., it was shown that at the end of the 7-week experimental period, the Bifidobacterium pseudocatenulatum and Bifidobacterium longum slightly decreased the concentration of Total cholesterol, LDL-cholesterol and Triglycerides in rats [20]. In another study, Sadeq et al. showed that the yoghurt containing Bifidobacterium pseudocatenulatum G4 or Bifidobacterium longum BB536 can reduce the concentration of serum cholesterol, LDL- cholesterol and Triglycerides but increased HDL-cholesterol in rats [21].

Cholesterol reduction by lactic acid bacteria can be explained by many mechanisms: which contains enzymatic deconjugation of bile acids by bile- salt hydrolase of Probiotics [22]. Assimilation of cholesterol by Bifidobacterum sp[23]. Co- precipiation of cholesterol with deconjugated bile by Lactobacilli strains [24]. Cholesterol binding to cell walls of Lactobcilli strains and Bifidobacteria[25]. Incorporation of cholesterol into the cellular membrance of Lactobacilli strains during growth [26]. Conversion of cholesterol into coprostanolby Lactobacilli [27] and production of short-chain fatty acides upon fermentation by Lactococcus lactis KF147 In the presence of prebiotics[28]. In addition, strong effects B.bifidum PTCC1644 of toward the reduction of triglyceride levels was shown in this study.

Table 3 serum levels of Total cholesterol, HDL – cholesterol and LDL – cholesterol ($\frac{mg}{dl}$) in rats fed experimental diet

	8-week-old	17-week-old
Total cholesterol		
SD	70.5 ±6.9	67.83 ±8.1
FSD	56.33 ±3.8[a]	80.33 ±10.9[a]
FSD-LAB	66.16 ±8.8	68.5 ± 9.6
LDL-cholesterol		
SD	12.00 ±2.2	13.66 ±2.2
FSD	10.66 ±1.3[a]	38.00 ±10.2[a]
FSD-LAB	11.33 ±2.7[b]	25.66 ±8.5[b]
HDL-cholesterol		
SD	43.00 ±4.2	36.83 ±4.9
FSD	34.00 ±3.09[a]	24.00 ±5.1[a]
FSD-LAB	37.5 ±5.9	28.16 ± 8.2

[1]SD, standard dietand MRS broth($1\frac{ml}{day}$); FSD, standard diet mixed 1%cholesterol and MRS broth($1\frac{ml}{day}$); FSD-LAB, standard diet mixed 1%cholesterol and MRS broth containing Bifidobacterium bifidum $10^8 - 10^9 \left(\frac{cfu}{ml}\right)$once a day.

Each value represents theMean±SD of six rat. Means with similar small letters superscripts are significantly different (P<0.05).

Conclusion

The finding of this study indicated that B.bifidum PTCC1644 can effective in managing serum lipid profile and coronary heart disease by decreasing Total cholesterol, LDL-cholesterol and Triglyceride and slightly increasing HDL-cholesterol levels.

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