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Effect of *Lactobacillus paracasei* HII01 on lipid profile and blood parameters in hypercholesterolemic patients: A preliminary study

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ABSTRACT

Background: Probiotics are live microbes that when administered in suitable amounts, confer a health benefit on the host. Probiotics are considered as a complementary and supportive therapeutic agent for the treatment of several diseases and for the management of several disorders. Hypercholesterolemia is a major risk factor for the incidence of cardiovascular (CV) diseases. Several pharmacological and dietary approaches are proposed and used to improve the cholesterol profile. **Aims and Objective:** The objective of the current study was to study the effect of probiotic supplementation (*Lactobacillus paracasei* HII01) on high-density lipoprotein (HDL), and low-density lipoprotein (LDL), lipopolysaccharide (LPS), and immunoglobulin A (IgA) levels in hypercholesterolemic patients. **Materials and Methods:** Ten hypercholesterolemic subjects were supplemented with 12.5×10^{10} CFU of HII01 per day for 12 weeks. Blood samples were collected at baseline and after the intervention period. The LDL-C, HDL-C, LPS, and IgA content were assessed by outsourcing and ELISA methods. **Results:** After 12 weeks of intervention, HDL (from 61.14 ± 9.65 to 67.29 ± 7.99 mg/DL), LDL (from 141.57 ± 31.95 to 126.29 ± 36.23 mg/DL), IgA (446.31 ± 262.00 to 939.62 ± 758.52 ng/ml), and LPS (113.53 ± 40.07 to 72.78 ± 32.39 pg/ml) levels were significantly changed in the probiotic-supplemented group when compared to the baseline value. **Conclusion:** The supplementation of *L. paracasei* HII01 may help to improve the lipid profile, reduce the endotoxin level and have the impact of immune activation in hypercholesterolemic subjects. Additional studies are mandatory to disclose the favorable effect of the probiotic strain *L. paracasei* HII01.

Key words: Probiotics; *Lactobacillus paracasei;* Hypercholesterolemia; High-Density Lipoprotein; Low-Density Lipoprotein

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INTRODUCTION

Dyslipidemia has been documented as a noticeable riskfactor for the development of cardiovascular (CV) diseases.[1] The guidelines and studies showed that statin as a main therapeutic agent to lower the low-density lipoproteincholesterol (LDL-C).[2] Lowering of high-density lipoprotein cholesterol (HDL-C) is an independent forecaster of CVdiseases.[3] Besides, meta-regression study suggested that the increase in HDL-C not necessarily improved the CV outcome.[4] The supplementation of 39 mg of statin reduced about 20 percent of the CV diseases risk, but the residual relative risk was not significantly nullified by the statin treatment.[5-7]

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Lifestyle changes that include the consumption of low fat and low calories food and regular physical activities are associated with weight loss and improved the lipid profile, and reduced the risk of CV diseases. Nevertheless, the lifestyle modifications are not sufficient to lower the LDL-C, and that could not be a primary therapeutic procedure to manage the CV diseases. So additional pharmacological approaches are needed to address the issues of dyslipidemia and risk of CV diseases.[8]

Probiotics are live microorganisms that when consuming in an adequate amount, confers health benefits to the host. Lactic acid bacteria (LAB), Gram-positive microbes are the most generally used probiotic strains, which have been used for several decades in food production. In recent years, screening of potent probiotics, development of probiotics-based alternative and complementary therapeutic strategies and extend the application of probiotics in possible scientific field have been increased. Probiotics, probiotic-based foods, and health supplements were reported for their positive beneficial effects on metabolic disorders,[9,10] antibiotic-associated diarrhea,[11] inflammatory bowel diseases,[12] skin diseases,[13] menopausalsymptoms,[14] mental health and cognitive improvement,[15] and the aging process.[16] In addition, LAB strains can be used as an effective microbial factory for the production of food-grade proteins and enzymes.[17]

Several *in vitro*, *in vivo* and human studies, described the cholesterol-lowering activity of probiotic interventions.[18] The supplementation of *Lactobacillus plantarum* PH04 lowered the triglyceride level and improved the cholesterol profile in hypercholesterolemic mice model.[19] Likely, another strain of *L. plantarum* KCTC3928 also exhibited cholesterol-lowering activity in C57BL/6 mice via modulating the bile acid excretion.[20]

The objective of the current study was to evaluate the cholesterol-lowering property of *L. paracasei* HII01 in hypercholesterolemic human subjects. The changes in HDL-C, LDL-C, lipopolysaccharide (LPS), and immunoglobulin A (IgA) level before and after the supplementation of *L. paracasei* HII01 have been reported in the study.

MATERIALS AND METHODS

Subjects and Intervention

The hypercholesterolemic subjects were screened and 10 people were selected. The study procedure has been explained thoroughly to the subjects and written concern agreement was made. The demographic information of the subjects was detailed in table 1. The probiotic strain *Lactobacillus paracasei* HII01 was cultivated and processed as live lyophilized powder at Lactomason Co., Ltd., South Korea. The aluminum foil sachet containing *L. paracasei* HII01 (12.5×10^{10} CFU/g) strain was supplemented to the volunteers for 4 weeks (one sachet per day) and asked them to follow their routine lifestyle. Mae Fah Luang University ethical committee evaluated and approved the study protocol (No. REH-6215; dated 14 June 2019).

Sample collection

Blood samples were collected at baseline and after 4 weeks of intervention in a sterile blood collecting tube and stored on ice or 4°C until analysis.

Measurement of HDL, LDL, IgA, LPS

HDL and LDL were measured at Associated Medical Sciences Clinical Service Center, Chiang Mai University. Immunoglobulin A (IgA) level was determined using Elabscience Human IgA ELISA Kit and the amount of lipopolysaccharide (LPS) was determined using MyBioSource human lipopolysaccharides ELISA Kit as per the manufacturer's instructions.

Statistical analysis

Data were analyzed using STATA15. Paired-sample t-test was used to detect within-group differences in lipid concentrations, IgA and LPS.

RESULTS

Ten hypercholesterolemic subjects (3 male and 7 female) were selected for the study. The average age of the subjects was 43.40 ± 16.37 years at the time of enrolment for the study. The subjects are non-diabetic and non-smokers but 20% of the subjects have the habit of drinking alcoholic beverages (Table 1).

Table 1: Demographic information of the volunteers

Characteristic	Probiotics (n=10) Number(Percentage)
Gender	
Male	3(30.0)
Female	7(70.0)
Age	
≤30	3(30.0)
31-59	6(60.0)
60 or more	1(10.0)
Mean±SD	43.40±16.37
Diabetes	
Yes	0
No	10(100.0)
Smoking	
Yes	0
No	10(100.0)
Alcohol drinking	
Yes	2(20.0)
No	8(80.0)

Change in HDL-C and LDL-C level

The LDL and HDL levels were recorded at baseline and after 4 weeks of *L. paracasei* HII01 supplementation. The level of LDL and HDL at baseline (Pre-treatment) was 156.80 ± 35.94 and 56.90 ± 10.65 mg/DL, respectively. Whereas after 4 weeks of probiotic intervention, level of LDL and HDL were changed to 139.40 ± 36.41 and 62.90 ± 9.90 mg/DL, respectively (p< 0.01) (Figure 1 and 2). After the probiotic intervention, a significant increase in HDL and a decrease in LDL levels were observed.

Change in IgA and LPS content

The concentrations of IgA and LPS were altered in hypercholesterolemic subjects after the probiotic supplementation. The average of 446.31 ± 262 and 939.62 ± 758.52 ng/ml of IgA values was observed at baseline and after the probiotic intervention, respectively (p = 0.02) (Figure 3). The baseline and after treatment of values of LPS was 113.53 ± 40.07 and 72.78 ± 32.39 pg/ml, respectively (p< 0.01) (Figure 4).

DISCUSSION

The probiotic-based interventions are recently considered as a booster for the pharmacological agents to manage dyslipidemia. The changes in food habits that includesoy and other legumes, colored rice, red mold rice, vitamin-C, omega-3 fatty acids, and nicotinic acid in the regular diet can improve the lipid profile.[21-26] Several clinical trials showed the beneficial effect of probiotic supplementation on the health status of hypercholesterolemic subjects.

Two-week supplementation of yogurt containing probiotic strains *Bifidobacterium lactis* and *Lactobacillus acidophilus* reduced the total cholesterol value while other lipid profiles were not affected significantly in healthy volunteers.[27] The intervention of *L. plantarum* strain mixture, in the form of a capsule, for 12 weeks effectively reduced the total cholesterol, LDL-C, LDL-D/HDL-C ratio, and triglyceride levels in hypercholesterolemic subjects.[28] Although, the meta-analysis of several randomized controlled clinical trials suggested that the supplementation of probiotic formulations decreased the triglycerides, LDL-C, and total cholesterol, and increased the HDL-C content when compared to the placebo control and baseline values.[29,30]

It is the truth that not all the probiotic interventions improved the lipid profile and other related parameters in human volunteers. The intervention of probiotic yogurt (made with *L. acidophilus* La5 and *B. lactis* Bb12) and

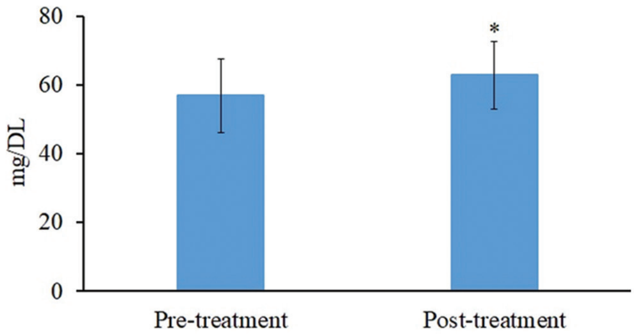

Figure 1: Changes in the level of HDL after 4 weeks of probiotic intervention in hypercholesterolemic patients. *indicates the significant difference between samples (p< 0.01).

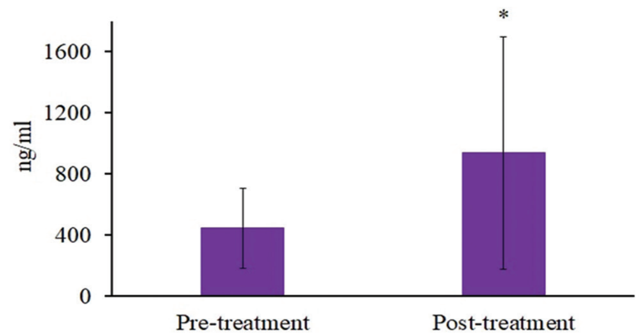

Figure 3: Changes in the level of IgA after 4 weeks of probiotic intervention in hypercholesterolemic patients. *indicates the significant difference between samples (p< 0.02).

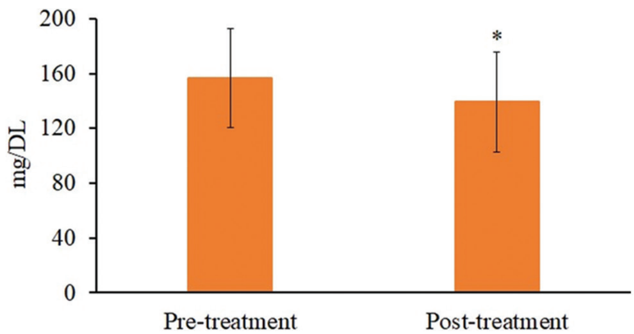

Figure 2: Changes in the level of LDL after 4 weeks of probiotic intervention in hypercholesterolemic patients. *indicates the significant difference between samples (p< 0.01).

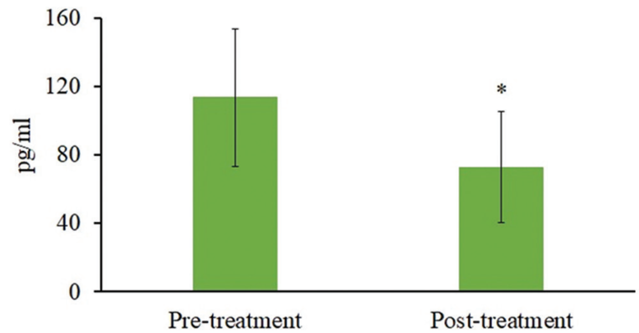

Figure 4: Changes in the level of LPS after 4 weeks of probiotic intervention in hypercholesterolemic patients. *indicates the significant difference between samples (p< 0.01).

consumption of kefir were not improved the lipid profile of the subjects.[31,32]

Ecologic® Barrier (Mixture of *B. bifidum* W23, *B. lactis* W52, *L. acidophilus* W37, *L. brevis* W63, *L. casei* W56, *L. salivarius* W24, *Lactococcus lactis* W19 and *L. lactis* W58) supplementation significantly reduced the blood endotoxin level and improved the lipid profile in diabetic patients.[33] Likely, the supplementation of the probiotic mixture (*L. acidophilus* T16, *B. bifidum* BIA-6, *B. lactis* BIA-6, and *B. longum* LAF-5) with or without prebiotics (maltodextrin) reduced the endotoxin level and improved inflammatory markers in hemodialysis patients.[34]

The results of the current study supported that the supplementation of lyophilized *L. paracasei* HII01 amended the lipid profile in hypercholesterolemic patients. Particularly, the consumption of HII01 significantly increased the HDL level (p< 0.01) (Figure 1) and decreased the LDL level (p< 0.01) (Figure 2).

The IgA level was significantly (p< 0.02) increased after intervention, but the standard deviation value was not significant (Figure 3). Thus, the result of this particular parameter could not be considered as an acceptable outcome of the probiotic intervention. Further experimental evidence is needed to support the immune modulatory activity of the *L. paracasei* HII01 strain in hypercholesterolemic subjects.

The LPS concentration was reduced drastically after the probiotic intervention, which indicates that the supplemented probiotics improved the hot gut-microbiota, and reduced the release of endotoxin in the bloodstream. The results suggested that the supplementation of *L. paracasei* HII01 could diminish the toxicity in the blood (Figure 4).

CONCLUSION

As per our knowledge, this is the primary study on the cholesterol-lowering property of *L. paracasei*. The results suggested that the consumption of 12.5 × 10^{10} cells of *L. paracasei* HII01 per day for 12 weeks significantly improved the blood lipid profile, endotoxin and IgA levels in hypercholesterolemic patients. Nonetheless, the current study has some downsides include limited sample size, no-placebo control, and limited studied parameters. Thus, a further extended study is obligatory with additional parameters to confirm the results of the present study.

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Authors Contribution:
CC- Concept and design of the study, manuscript preparation, critical revision of the manuscript; **BSS-** Statistically analyzed and interpreted, manuscript preparation, critical revision of the manuscript; **SS-** Concept and design of the study, statistically analyzed and interpreted; **YT-** Concept and design of the study, statistically analyzed and interpreted; **PS-** Manuscript preparation, critical revision of the manuscript

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