ISSN Online: 2456-4192

Anti-hypercholesterolemia and Anti-hyperlipidemic Effect of *Lactobacillus plantarum and Lactobacillus acidophilus* in Diabetic Rats

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Received 02 May 2017; accepted in revised form 11 June 2017

Abstract: This study was aimed to determine the cholesterol lowering and maintaining lipid profile effects of *Lactobacillus* strains in alloxan induced diabetic rats. Diabetic rats were treated with *Lactobacillus plantarum* and *Lactobacillus acidophilus* (10^8 CFU/ml) orally for 2 h or 21 days followed by measuring body weight and serological biomarkers such as triglyceride, total cholesterol, HDL, LDL, VLDL, TC/HDLC ratio and LDLC/HDLC ratio. After the administration of *Lactobacillus* strains for 21 days TG, TC, VLDL cholesterol and LDL cholesterol levels were significantly ($P < 0.001$) increased, whereas HDL cholesterol was decreased in diabetic rats compared to normal rats. Both the *Lactobacillus* strains exhibited significant reduction ($P < 0.001$) in all tested lipid and body weight parameters and restored them to nearly-normal values. The findings confirm anti-hypercholesterolemia and anti-hyperlipidemic efficacy of *Lactobacillus* spp.

Key words: *Lactobacillus* spp., total cholesterol, triglycerides, anti-diabetic.

Introduction

Cholesterol plays a critical role in cell, tissue and hormone formation. Food containing trans-saturated fat may elevate the level of cholesterol leading to a high risk of cardiovascular diseases. Access deposition of cholesterol on the blood vessels (Fig. 1) causes the formation of hard-lumps called plaque which may result in the various major complication in blood vessels such as narrowing, hardening, and decrease in blood flowing eventually causing heart diseases and hypertension [3]. Low-density (LDL) and high-density (HDL) lipoproteins major participate in the formation of cholesterol, whereas, higher levels of triglycerides may contribute to plaque formation [8]. In the USA, diabetic population has higher rates of cholesterol abnormalities (low levels of HDL and higher levels of triglycerides), and this contributes to the higher rates of heart disease among the diabetic people [9].

Probiotics are defined as live microorganisms which when administered in adequate amounts confer a health benefit on the host which have significant ability to lowering down the levels of cholesterol [7]. The lactic acid bacteria (LAB) present in the intestine are considered probiotic bacteria which have shown enormous potential on human health as well as exhibit various

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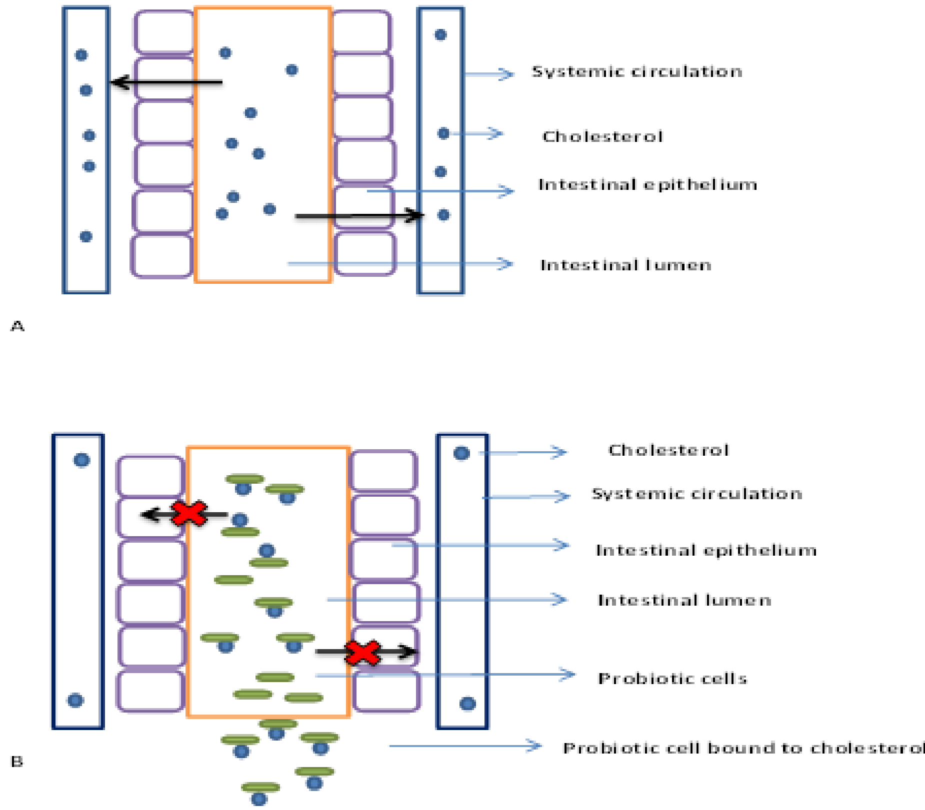

Fig. 1. Schematic representation of role of probiotic bacteria in cholesterol assimilationmechanism. (A) Absorption of cholesterol by the intestinal epithelium (B) Administration of probiotic bacteria enhances cholesterol assimilation, leading to the excretion of nonmetabolized cholesterol and other lipid molecules.

biological activities including immunomodulating and anticancer potential [6].

Recent findings have confirm lipid and cholesterol reducing ability of probiotic bacteria [5] mediated through the activity of bile-salt hydrolase [4]. On the other hand, assimilation of cholesterol by probiotic bacteria results in the reduction of luminal cholesterol. A diet rich with probiotics could be considered an alternative strategy for combating diabetes, and obesity like diseases [10].

The present study was designed to investigate *in vivo* anti-hypercholesterolemia and anti-hyperlipidemic effect of *Lactobacillus plantarum* KP894100 and *Lactobacillus acidophilus* KP942831 in alloxan-induced diabetes in Wistar rats in terms of estimation of serum cholesterol, serum triglycerides, HDL cholesterol, LDL cholesterol, VLDL cholesterol, TC/HDLC, and LDL/HDLC.

Materials and methods

Bacterial suspension preparation

Lactobacillus strains used in this study were previously isolated from milk and different dairy products such as cheese, yogurt, and curd and identified as *Lactobacillus plantarum* KP894100 and *Lactobacillus acidophilus* KP942831 [2]. The cultures were incubated in 400 ml of de Man Rogosa Sharpe broth (MRSB) at 72 h, aliquots were transferred to tubes for taking optical density (OD) at 600 nm until the OD reaches to 0.1 nm. According to McFarland standard in 0.1 nm OD is equivalent to 10^8 cfu/ml of bacterial culture.

Animals

Wistar rats of either sex (weighing 200-300 g) were maintained in a well-ventilated room, fed on commercial balanced pellet feed, distilled water, *ad-libitum* and under standardized ethical conditions. All studies on animals were approved by the Institutional Animal Ethics Committee (IAEC) Department of Pharmaceutical Sciences, Dr. Hari Singh Gour University, Sagar Madhya Pradesh, India.

Alloxan induced rats

The acclimatized rats were starved for 24 h with water *ad libitum* and diabetes was induced in rats by intraperitoneally injection of freshly prepared alloxan monohydrate (40 mg/kg b.w.) [11]. Animals with blood sugar level (200-350 mg/dl) were considered diabetic and were used in this study.

Experimental groups

After 48 h of the induction of diabetes, animals were randomly divided into 7 groups where each group consisted 6 rats. Animals were fed with standard diet on a regular basis, and simultaneously received probiotic bacterial culture with distilled water as follows: Group 1 served as a normal control (NC) which received standard diet and only distilled water. Group 2 served as a hyperglycemic control (HC) without treatment, whereas group 3 was served as positive control (PC) with a treatment of standard antidiabetic drug glibenclamide (50 mg/kg b.w.). Group 4 and 5 orally received 10^8 cfu/ml of *Lactobacillus plantarum* KP894100 (LP) and *Lactobacillus acidophilus* KP942831 (LA), respectively. All treatments were followed for 21 days.

Estimation of biochemical parameters

Blood samples were collected from the overnight fasted animals by retro-orbital puncture under light ether anesthesia at days 14 and 28 post-medication and preserved into blood sampling vials containing sodium fluoride followed by centrifugation at 3,500 rpm for 15 min to separate the plasma. The insulin levels were determined through a radio-immunoassay procedure, using insulin kits according to the manufacturer's instructions. At the end of the experiment, two blood samples were withdrawn from the overnight fasted animals into heparinized tubes. The first blood sample was used for estimation of total Hband HbA1c [15]. On the 21st day of the therapy, animals were fed with the respective bacterial cell, fasted for 3 h and blood samples (1 ml) were collected into EDTA containing bottles from the retro-orbital fluxes. Blood samples were allowed to clot and serum was separated by centrifugation (2500 rpm/15 min). The biochemical analysis of serum was done by using standard commercial kits according to the manufacturer's protocol. Serum total cholesterol (STC), serum triglycerides (STG), and serum high density lipoprotein-cholesterol (HDL-C) were measured using kits from Agappe Diagnostics Ltd. (India). The TC/HDL-C and LDL-C/ HDL-C ratios markers of dyslipidemia were also determined. The VLDL cholesterol and LDL-cholesterol in serum were calculated as follows: Low density lipoprotein-cholesterol (LDL Cholesterol): VLDL cholesterol= TG / 5; LDL-cholesterol = TC - TG / 5 - HDL-cholesterol.

Statistical analysis

Data were calculated as mean with standard error. One way ANOVA was employed for statistical analysis with a significant level if P is < 0.05, < 0.001< 0.0001.

Results

Effect of Lactobacillus spp. on body weight of animals

The results of the effect of both *Lactobacillus* bacterial cultures at 10^8 cfu/ml /day dose on body weight of alloxan monohydrate induced diabetic rats after 21 days of treatment are presented in Fig. 2. Body weight of normal control rats was increased during the period of the experiment, while from the 3rd day of the experiment diabetic group showed decreases in body weight. The body weight of other groups treated with *Lactobacillus* strains was ascended. The body weight of the rats treated with 10^8 cfu/ml /day *L. acidophilus* was ascending similar to that of normal control, and 10^8 cfu/ml /day *L. plantarum* groups take the third and fourth order in body weight increase.

Effect of *Lactobacillus* spp. on serum lipid profile

The administration of *Lactobacillus* strain for 21 days TG, TC, VLDL cholesterol and LDL cholesterol levels were significantly (P < 0.001) increased, whereas HDL cholesterol was decreased in diabetic rats compared to normal rats (Table 1). The ratio of TC/HDL cholesterol and

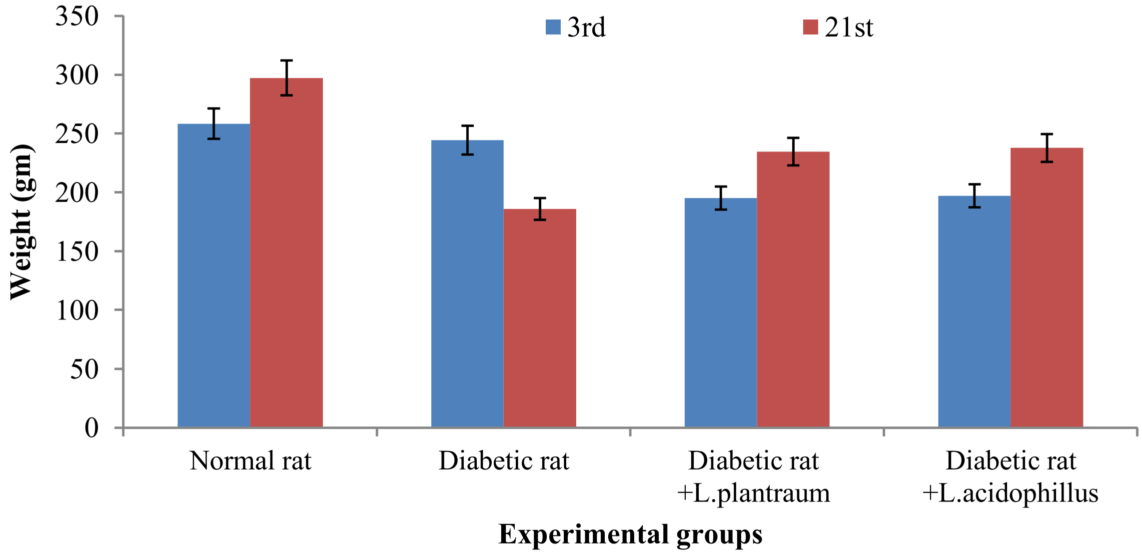

Fig. 2. Effect of Effect of lactobacillus strains on body weight of diabetic rats after treatment on 3rd, and 21st day. Values are expressed as mean ± SE (n = 6)

Table 1. Effect of *Lactobacillus* bacteria on serum lipid profile in alloxan induced diabetes in Wistar rats

Biochemical	Normal rat	Diabetic rat	Positive control	*L. plantarum*	*L. acidophilus*
Total Cholesterol (mg/dl)	88.71±0.4	147.73±2.45	113.15±4.65	126.65±8.25	121.15±0.26
Total Triglycerides (mg/dl)	108.06±1.2	212.94±0.63	126.45±3.16	136.69±4.32	131.35±0.12
HDL Cholesterol (mg/dl)	29.72±1.26	24.09±01.67	31.93±4.87	37.87±1.46	34.93±0.14
LDL Cholesterol (mg/dl)	37.378±0.65	80.51±1.34	55.87±0.58	61.442±2.69	59.95±0.32
VLDL Cholesterol (mg/dl)	21.612±2.87	42.588±2.56	25.29±0.64	27.27±0.85	26.27±0.25
TC/HDLC ratio	2.984±0.94	6.134±6.25	3.543±1.84	3.344±0.63	3.468±0.32
LDLC/HDLC ratio	1.257±1.35	3.342±4.65	1.749±1.67	1.622±0.11	1.716±0.32

HDL: High-density lipoprotein
LDL: Low-density lipoprotein

LDL cholesterol/HDL cholesterol (markers of dyslipidemia) was significantly raised in the diabetic group. The HDL cholesterol value was significantly increased by both bacterial cells at 10^8 cfu/ml. Both the *Lactobacillus* strains exhibited significant reduction (P<0.001) in all tested lipid parameters and restoring them to nearly-normal values.

Discussion

Probiotic lactic acid bacteria (LAB) have shown enormous potential in the treatment of various diseases, including diabetes and obesity [6]. The probiotic strains used in this study exhibited profound antidiabetic effect as confirmed by the reduction in the body weight of test animals when orally administered by the *L. plantarum*

KP894100 and *L. acidophilus* KP942831. From 7th day, the body weight of diabetic rats was continuously reduced as compared to the normal control rats. Glibenclamide as a positive control, improved the body weight of diabetic rats with a significant increase (21%) ($P < 0.05$) compared to the diabetic control rats. The *L. plantarum* and *L. acidophilus* (10^8cfu/ml) significantly reduced ($P < 0.001$) the body weight of diabetic rats by 26% when compared to diabetic control rats. In addition, it was also observed in this study that both the probiotic strains *L. plantarum* and *L. acidophilus* were able to increase the levels of TC, TG, LDL, and VLDL whereas decrease the levels of HDL in diabetic animals. Total cholesterol, triglycerides, LDL, and VLDL of the alloxan induced diabetes rats treated with *Lactobacillus* culture (10^8 cfu) showed the significant reduction, and improved the level of HDL cholesterol as compared to diabetic rats.

It was observed in this study that probiotic bacteria have the ability to reduce cholesterol, glucose and high lipid concentration. It has been suggested that there is a positive correlation between HDL and heart diseases, however, LDL: HDL cholesterol ration also contributes to such diseases [13].

Wang *et al.* [14] stated that probiotic bacteria such as *L. plantarum* CAI6 and *L. plantarum* SC4 may protect against cardiovascular disease by the regulation of lipid metabolism and induced antioxidative defense in hyperlipidemic mice. Also Alsayadi *et al.* [1] reported anti-hyperglycemic and anti-hyperlipidemic activities of probiotic strain in diabetic rats. Further, Shrivastava *et al.* [12], also reported the lipid lowering and antioxidant effect of aqueous suspension of *L. acidophilus* (10^9 CFU/ml) on high fat diet fed rats. They found a significant decrease in plasma cholesterol, triglycerides, and LDL as well as an increase in HDL levels.

Conclusion

In this study, *Lactobacillus* strains *Lactobacillus plantarum* KP894100 and *Lactobacillus acidophilus* KP942831 exhibited significant anti-hypercholesterolemia and anti-hyperlipidemic effects in animal model, and were able to influence various biochemical parameters and body weight in tested diabetic animals. Based on the findings of this study, it can be suggested that probiotic strains *L. plantarum* KP894100 and *L. acidophilus* KP942831 used in this study could be potential candidates for your using as anti-diabetic agents. Further strategies on formulation studies in the laboratory are warranted.

Acknowledgements

This work was supported by a grant from the University grant commission, New Delhi, India. The authors would like to thank the Department of Pharmaceutical Sciences, Dr. H.S. Gour University Sagar M.P. India for providing help to do animal and animal house also.

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