Academic Sciences Asian Journal of Pharmaceutical and Clinical Research

Vol 6, suppl 2, 2013 ISSN - 0974-2441

Research Article

HYPOLIPIDEMIC AND ANTIOXIDATIVE EFFECT OF *LACTOBACILLUS ACIDOPHILUS* BACTERIA IN HYPERLIPIDEMIC RATS.

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Received: 13 December 2012, Revised and Accepted: 17 January 2013

ABSTRACT

Accumulation of cholesterol and low density lipoprotein in excess amount is hallmark of atherosclerosis plaque progression. Furthermore oxidative stress also increases the risk of plaque formation. In the present study, aqueous suspension of *Lactobacillus acidophilus* (2X10^9 CFU/mL) was studied to evaluate its lipid lowering and antioxidant effect on high fat diet fed Charles foster rats. Feeding of *Lactobacillus acidophilus* caused a significant decrease in plasma cholesterol (17%), triglycerides (20%) and LDL (33%) as well as a remarkable increase in HDL (42%) levels. These effects were resulted from the activation of lecithin: cholesterol acyltransferase and post heparin lipolytic activities. Moreover enhanced excretion of faecal bile acids is also responsible for low plasma cholesterol levels. *Lactobacillus acidophilus* also showed potent antioxidant effect as it significantly reduces lipid peroxidation and normalizes the activities of antioxidant enzymes.

Keywords: Hyperlipidemia, *Lactobacillus acidophilus*, Oxidative stress, Antioxidants

INTRODUCTION

The involvement of dyslipidemia in development of macro-/microvascular complications viz., cardiovascular disease (CVD), cerebro-vascular disease and peripheral vascular disease, which account for more than 70% of deaths in individuals with diabetes mellitus[1]. When plasma cholesterol exceeds the level required, it results in the development of atherosclerosis and stroke[2]. The treatment of dyslipidemia reduces cardiovascular events[3]. The modern pharmacological therapy for hyperlipidemia is effective but associated with side effects leading to patient incompliance[4]. Moreover, the lipid lowering drugs viz. fibrates, statins and bile acid sequestraints do not possess antioxidant property[5]. Therefore, there is a need of a therapy having dual property of hypolipidemic and antioxidant effects. For this purpose microorganisms would be the most preferred option as many bacteria are the natural inhabitants of human body.

Lactobacillus acidophilus is a bacterium which is naturally found in human gastrointestinal tract, mouth and vagina. It has an optimum growth temperature 37˚c and pH below five[6]. L. acidophilus is able to survive gastrointestinal transit, being resistant to bile, low pH, and digestive enzymes[7]. They may then be able to adhere to human epithelial cell lines and human intestinal mucus. Several studied have reported that lactic acid bacteria fermented food show hypolipidemic effects by inhibiting cholesterol biosynthesis and decreasing low density lipoproteins[8, 9]. The present study was designed to investigate the hypolipidemic and antioxidative effect of Lactobacillus acidophilus on high fat diet fed hyperlipidemic Charles foster rats.

MATERIALS AND METHODS

The bacterium

The spores of *Lactobacillus acidophilus* was purchased from local chemist shop and cultured in MRS agar. The bacterium was identified according to Gram stain positive and Catalase negative. The water suspension of *L. acidophilus* (LAS) was prepared in sterile triple distilled water containing 2X10^9 CFU/ml.

Animals

Male adult rats of *Charles Foster* stain (100-150g) bred in the animal house of Central Drug Research Institute, Lucknow (India). The animals were used after approval of Institutional Animal Ethics Committee.

Chemicals

The kits for total cholesterol (TC), triglyceride (TG), HDL, and all other chemicals were procured from Sigma Chemical Company St Luis, MO (USA).

Experimental design

The animals were randomly divided into five groups; group 1 – normal control, group 2 – HFD fed, group 3 – HFD with LAS (1ml), group 4 – LAS with normal diet fed rats and group 5 – HFD fed with standard drug Gemfibrozil (100mg/kg b. w.). The LAS was given orally, simultaneously fed with high fat diet for 30 days[10]. At the end of treatment the animals were fasted for 24 hours, the blood of anesthetized animals was collected by cardiac puncture in EDTA coated glass tubes and centrifuged at 2500Xg for 10min in Sigma 3-30k centrifuge to obtain plasma. The animals were sacrificed to collect liver tissue in ice cold glass tubes.

Biochemical analysis of plasma

The levels of total cholesterol (TC), triglycerides (TG), phospholipids (PL), high density lipoproteins (HDL) and post heparin lipolytic activity (PHLA) & Plasma lecithin: cholesterol acyltransferase (LCAT) activity were estimated according to methods mentioned[11-16]. VLDL and LDL were calculated according to following formulas[17].

$$[VLDL = TG/5]; [LDL = TC - HDL - TG/5]$$

Measurement of faecal excretion of bile acids

Faeces of each group were collected daily. The amount of cholic acid and deoxycholic acid excreted through faeces was estimated[18].

Antioxidant activity

Lipid peroxidation in plasma was measured by Thio-Barbituric acid reaction as TBARS[19]. Hepatic Superoxide dismutase (SOD), Glutathione peroxidase (GPx), and Glutathione reductase (GRh) were estimated by methods reported earlier[20-22].

Statistical analysis

All groups were compared by one way analysis of variance (ANOVA) & the significance of mean difference between different groups was done by Tukey's post hoc test. A two tailed (α=2) probability $p<0.05$ was considered statistically significant ($p < 0.05$ = *, $p < 0.01$ = **, $p < 0.001$ = *** and ns = not significant).

RESULTS

Lipid Profile

The chronic feeding with HFD caused a marked increased in plasma levels of TC (+2.4 fold), TG (+2.6 fold), PL (+2.1 fold), LDL (+6.3 fold) and VLDL (+2.6 fold) as well as a decrease in HDL (-38%). These effects were shown to be reversed by the treatment with LAS by 17%, 20%, 11%, 33%, 20% and 42% respectively (Figure 1).

Figure 1: Plasma Lipid Profile

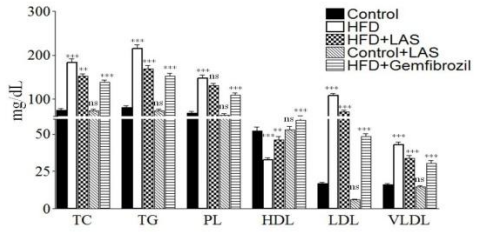

Figure 1: Biochemical analysis of plasma of HFD induced hyperlipidemic animals. Effect of LAS on lipid profile was studied. Control group was compared with HFD fed; LAS treated with HFD rats. All groups were compared by one way analysis of variance (ANOVA) p < 0.05 = *, p < 0.01 = **, p < 0.001 = * and ns = not significant.**

Lipolytic enzymes

HFD feeding caused a significant decrease in activities of LCAT (30%) and PHLA (34%) in plasma Treatment with LAS significantly reactivated these lipolytic activities by 13 & 29% respectively in hyperlipidemic rats (Figure 2).

Figure 2: Lipolytic Enzyme

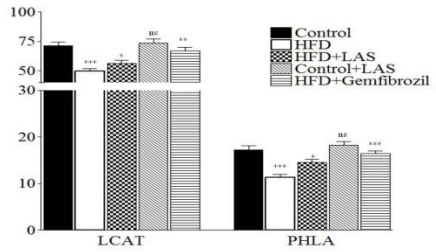

Figure 2: Lipolytic enzymes. Control group was compared with HFD fed; LAS treated with HFD rats. Units, n mol cholesterol released/h/L for LCAT and n mol free fatty acid formed/h/ml for PHLA. All groups were compared by one way analysis of variance (ANOVA) p < 0.05 = *, p < 0.01 = **, p < 0.001 = *** and ns = not significant.

Faecal excretion of bile acids

Feeding with HFD caused a significant decrease in the faecal excretion of cholic acid (32%) and deoxycholic acid (33%) and these levels were shown to be recovered by the treatment with LAS by 25 & 20% respectively (Figure 3).

Figure 3: Faecal Bile Acids

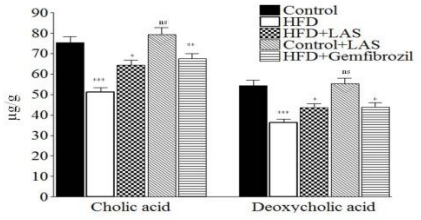

Figure 3: Effect of LAS on bile acid excretion. Excretion of cholic acid and deoxycholic acid (unit, µg/g) was found to significantly be increased in LAS treated animals compared with untreated animals fed on HFD. All groups were compared by one way analysis of variance (ANOVA) p < 0.05 = *, p < 0.01 = **, p < 0.001 = *** and ns = not significant.

Antioxidant activity

Due to the chronic feeding of HFD plasma levels of TBARS significantly increased (+2.1 fold) which was reduced by LAS (25%). The total activities of SOD, GPx & GRh were found to be higher in HFD fed animals than those fed on normal diet. The feeding of LAS significantly normalized these effects (Figure 4).

Figure 4: Antioxidant Effect

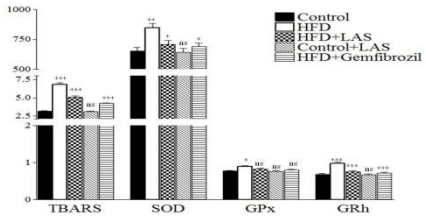

Figure 4: Effect of LAS treatment was observed on lipid peroxidation as TBARS (unit, nmol malonaldehyde /ml) in plasma as well as SOD (superoxide dismutase), GPx (glutathione peroxidise) and GRh (glutathione reductase) in liver (Units: SOD units are expressed as amount of enzyme that inhibits the formation of formazone by 50%; GPx: µmole NADPH oxidized / min/ ml; and GRh: µmole NADPH oxidized / min/ ml). All groups were compared by one way analysis of variance (ANOVA) p < 0.05 = *, p < 0.01 = **, p < 0.001 = *** and ns = not significant.

DISCUSSION

Atherosclerosis is an emphatically serious condition where medium and large arteries become clogged up by fatty substances results in formation of plaques. Disorders of lipid and lipoprotein metabolism *i.e.* dyslipidemia are traditional risk factors for atherosclerosis. Accumulations of cholesterol and LDL are main cause for formation of atherosclerotic plaque which results in strokes, heart attack and eventually death[23]. High level of cholesterol increases oxidative stress by production of endothelial superoxide anions, results in oxidative modification of LDL-associated lipids[24]. The oxidized LDL is directly involved in the initiation of atherogenesis[25]. Increased plasma cholesterol, LDL and decreased HDL together comprise atherogenic phenotype[26]. Two types of therapies are generally used for treatment of atherosclerosis targeting either oxidative stress or cholesterol biosynthesis. Antioxidant based therapies are designed to reduce reactive oxygen species and oxidation of LDL. HMG-CoA-reductase inhibitors (statins) are drugs of choice for targeting cholesterol biosynthesis. However, statins do not possess antioxidant properties, furthermore some recent studies indicates that they cause an adverse effect on neurotransmission in brain[27]. Moreover, several reports have shown that a combination therapy is better than mono-therapy[28]. In search of a better therapy option with dual property of antioxidant and lipid lowering activity, the live suspension of *Lactobacillus acidophilus* was studied in HFD induced hyperlipidemic rats.

High fat diet induces endothelial dysfunction, atherosclerosis and increases oxidative stress by increasing the expression of oxidation-sensitive genes[29, 30]. The present investigation shows that LAS significantly increases the activity of LCAT, which plays a key role in lipoprotein metabolism. LCAT converts free cholesterol into cholesteryl ester (a more hydrophobic form) which is then sequestered into the core of alipoprotein particle, eventually making the newly synthesized HDL. Therefore activation of LCAT lowers cholesterol levels and increases levels of HDL. The post-heparin lipolytic activity of plasma mainly consists of two activities: triglyceride lipase and lipoprotein lipase, and inhibition of PHLA results in elevated levels of TG, PL and VLDL[31]. Therefore activation of PHLA is responsible for a significant decrease in levels of TG, PL and LDL & VLDL. The increased excretion of faecal bile acids is also linked with cholesterol lowering effect of LAS. Since cholic acid is synthesized from cholesterol in liver, increased excretion of cholic acid results in decreased level of cholesterol. Akalin *et al* found that consumption of acidophilus yogurt significantly lowered the values for plasma TC, LDL-c in the mice[32]. Researchers showed that lactic acid bacteria (LAB) could decrease the level of cholesterol but the mechanism has not been demonstrated clearly yet. Some predicted that LAB, bile salt and cholesterol were co-precipitated, and then expelled with faeces, or the cholesterol was absorbed by LAB[33]. Otherwise, Smet *et al* suggested that the reason of LAB reducing cholesterol might be due to the activity of bile salt hydrolysis produced by the LAB[34]. Findings of our present study suggest that lipid lowering effect of LAB is due to activation of LCAT, PHLA and enhanced excretion of bile acids.

High fructose consumption has pro-oxidant effect. Fructose fed rats display oxidative stress, an imbalance between free radical

production and antioxidant defence in many tissues[35-37]. The lipid peroxidation was analyzed by TBARS. The Thio-Barbituric Acid (TBA) test is a very non-specific technique and is widely used as a marker[38]. LAS therapy lowered the levels of TBARS indicating decreases in lipid peroxidation. The elevated activities of antioxidant enzymes indicate high levels of oxidative stress. Several studies demonstrated that some lactobacilli possess antioxidative activity, and could decrease the risk of accumulation of reactive oxygen species during the ingestion of food[39, 40]. The antioxidant effect may be linked with the scavenging of reactive oxygen species and hydroxyl free radicals. The LAS normalized activities of superoxide dismutase, glutathione peroxidase and glutathione reductase.

Based on above findings, we can conclude that activation of LCAT & PHLA and increased excretion of faecal bile acids are together responsible for lipid lowering activity of *Lactobacillus acidophilus*. Its antioxidant and lipid lowering activities remarkably reduce risk of atherosclerosis. The data of present study showed that the *Lactobacillus acidophilus* will be a better therapy option for atherosclerosis and dyslipidemia as it possesses both antioxidant and lipid lowering effects.

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