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

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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 (2X10⁶ 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 keitin: cholesterol acyltransferase and post heparin lipolytic activity. 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 complication viz., cardiovascular disease (CVD), cerebro-vascular disease and peripheral vascular disease, which account for more than 70% of deaths in individuals with diabetes mellitus. When plasma cholesterol exceeds the level required, it results in the development of atherosclerosis and stroke. The treatment of dyslipidemia reduces cardiovascular events. The modern pharmacological therapy for hyperlipidemia is effective but associated with side effects leading to patient inconvenience. Moreover, the lipid lowering drugs viz. fibrates, statins and bile acid sequestrants do not possess antioxidant property. 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 bacteria which is naturally found in human gastrointestinal tract, mouth and vagina. It has an optimum growth temperature 37°C and pH below five. L. acidophilus is able to survive gastrointestinal transit, being resistant to bile, low pH, and digestive enzymes. 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. 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 bacteria

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 2X10⁶ 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. 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 keitin: cholesterol acyltransferase (LCAT) activity were estimated according to methods mentioned. VLDL and LDL were calculated according to following formulas.

\[ VLDL = TG/5 \]
\[ LDL = TC - HDL - TG/5 \]

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.

Antioxidant activity

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

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 of LAS by 17%, 20%, 11%, 33%, 20% and 42% respectively (Figure 1).
The present investigation shows that LAS 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).

**Lipolytic enzymes**

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).

**Faecal excretion of bile acids**

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).

**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. High level of cholesterol increases oxidative stress by production of endothelial superoxide anions, results in oxidative modification of LDL-associated lipids. The oxidized LDL is directly involved in the initiation of atherosclerosis. Increased plasma cholesterol, LDL and decreased HDL altogether comprise atherogenic phenotype. 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. Moreover, several reports have shown that a combination therapy is better than mono-therapy. 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. 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 allipoprotein 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. 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 mice. 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. 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. 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.
production and antioxidant defence in many tissues. 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. 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 antioxidant activity, and could decrease the risk of accumulation of reactive oxygen species during the ingestion of food. 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 & Dismutase, glutathione and could decrease the 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.

REFERENCE

