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ORIGINAL ARTICLE / RESEARCH

Systemic Immunomodulation and Hypocholesteraemia by Dietary Probiotics: A Clinical Study

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ABSTRACT

Probiotics intake has been associated with beneficial effects on the hyporcholesteremia and gut immune system. This improves disease resistance, gut infection and diminished risk were documented. In one of our experimental study in mice, probiotics was found to modulate the immune response and at the same time it decreased cholesteremia. The present study was extended to human beings, male healthy volunteers, to find out whether probiotics given in the form of fermented milk product *'Dahi'* and *'Lassi'* show similar effect i.e. enhanced immune response and hypocholesterolemia. The immune response was checked before and after feeding of probiotics by employing INOs and NBT reduction tests. In addition the effect of probiotics on hypertension and cholesteremia was also observed. The results show significantly enhanced immune response by probiotic consumption and non significant reduction in cholesterol level, HDL-C in the human subjects and decrease in systolic blood pressure in hypertensive patients by the consumption of *'Dahi'* and *'Lassi'* was also observed. It is suggested that probiotic diet therapy can be a safe additive or alternative to existing drug therapy.

Keywords: INOs; Inducible nitric oxide synthase, NBT; Nitroblue Tetrazolium Chloride, systolic blood pressure, immune response HDL-C; High Density lipoprotein cholesterol.

Introduction

The role of probiotics in human health is well documented now. There are reports which reveal that probiotics results in hypocholesteraemia [3],[21],[24],[31], ameliorate the gut immunity [5-7],[16],[30] and as a result are helpful in modifying diseases like atherosclerosis and gut infection. But the literature lacks the effect of probiotics on systemic immunity as well as mechanism of hypocholesteraemia. In one of our preliminary studies in mice a direct correlation was observed between immunomodulatory and hypocholesteraemic property of probiotics [10]. Not only this, the immune status of the host may modulate many immunological disorders and immune related diseases e.g. diabetes, cholesteraemia etc. The increase in awareness of the consumer about the side effects and cost of allopathic medicines has resurged the interest of the consumer in alternative therapy. The dietary materials are the most accepted alternative therapeutic agents. Hence the present study was designed to see the effect of consumption of probiotics in the form of '*Lassi*' and '*Dahi*', a common Punjabi food on systemic immunity and cholesteremia in human subjects. In addition the effect of probiotics consumption on hypertension was also observed.

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Materials and methods

DIET: '*Dahi*' and '*Lassi*' made by fermenting the low fat milk, containing *lactobacillus sps.,*

Streptococcus sp. were procured from Verka Milk Plant Ltd., Mohali and checked for proximate composition and viable cell count 2.1 × 10^8 $cfug^{-1}$ with routine microbiological methods were given to each subject to be consumed @ '*Lassi'* 350 ± 50 g/d/subject and '*Dahi'* 250 g/d/subject, for consecutive 30 days.

GROUPING:

For cholesterol level; Total sixty volunteers were divided in two group; Group-Ia (n=30) as control, the volunteers in this group were not recommended any test diet during the period and test Group IIa (n=30) on test diet for 30 days.

For Hypertensive study; Total 20 hypertensive patients attending OPD of Govt. Rajindra Medical College and Hospital and Amar Hospital, Patiala, Punjab, for regular blood pressure check up were divided into two Groups:

Group–Ib (n=10) (control hypertensive), subjects were not on test diet.

Group-IIb (n=10) (test hypertensive) The patients in this group were having hypertensive disease but were not taking any kind of medication. In addition to the normal diet, this group volunteers took *'Dahi'* and *'Lassi'* daily for 30 days as given above. No attempts were made to alter life style pattern of volunteers and they were told to maintain their usual habits as per the advice of the consultant doctor for both anti-cholesterolemic and hypertensive study [13].

Follow up of study

The study design was described to each volunteer. Each Group was instructed to follow diet guidelines as suggested by White et al. [13] and consume '*Lassi*' and '*Dahi*' for 30 days. Multiple blood samples were obtained from each Group on the zero day and 30 day of study and assayed for total serum cholesterol, HDL-C, inducible nitric oxide synthase (INOs) activity and Nitroblue tetrazolium chloride (NBT) reduction test (a measure of immune status).

Estimation of Total serum cholesterol:

Total serum cholesterol was estimated by the method of Wybenga et al. [1] using commercial kit (Diagnostic Reagent Kit manufactured by Span Diagnostic Ltd., India) based on reaction of cholesterol hot solution of ferric perchlorate, ethyl acetate and sulphuric acid, which gives lavender color complex which is measured spectrophotometerically at 540 nm.

The concentration of cholesterol in mg/dl of the test samples was calculated as:

Total Serum Cholesterol (mg/dl) = (O. D. of Test (T)/ O. D. of Standard (s)) x 200

HDL cholesterol (HDL-C): HDL-C was determined by one step method of Wybenga et al. [1] using commercial kit, manufactured by Span Diagnostic Ltd., India, Briefly 0.2 ml of serum sample mixed with 0.2 ml of precipitating reagent and based on reactions, lavender color complex which is measured by using UV-VIS spectrophotometer (Shimadzu) at 540 nm.

The concentration of serum HDL cholesterol in mg/dl of the test samples was calculated as:

Serum HDL-C (mg/dl) =(O. D. of Test (T)/ O. D. of Standard (s)) x 50

Determination of INOS (Inducible nitric oxide synthase test) activity: The iNOS activity was measured *in vitro* in blood lymphocytes (suspended in MEM @ 1 × 10^6 viable cells/ml) using arginine and Greiss reagent by the method of Stuehr and Marletta [11]. Optical density of the citrulline formed was determined spectrophotometerically with a UV-VIS spectrophotometer (Shimazu) at 540 nm against control.

Determination of Nitroblue Terazolium Test reduction (NBT): The NBT reduction test was employed to assess the function of phagocytes as described by Hudson and Hay [25]. Briefly, blood leukocytes were exposed to NBT and development of color due to formazon was measured spectrophotometerically at 520 nm using UV-VIS spectrophotometer, against dioxan as standard.

Percent NBT reduction = (Optical Density of test – Optical Density of control)/ Optical Density of control

Statistical analysis:

The primary objective was to assess the effect of fermented milk product *'Lassi'* and 'D*ahi'* on percentage change in serum cholesterol level, HDL-C, immune activity and systolic blood pressure on human subjects from day 0 to day 30. Statistical analysis was performed by student's t-test.

Results:

The results [Table/Fig 1] and [Table/Fig 2] show consumption of probiotic containing diet (*Dahi*' and '*Lassi*') in Test Group-IIa resulted in 10.30% ±1.32 reduction in total serum cholesterol and 3.56% ±0.8 difference in HDL-C after 30 days on test diet. These differences in total serum cholesterol and HDL-C were insignificant statistically by ($p<0.05$). The effect of probiotics consumption on immune response is shown in [Table/Fig 3], [Table/Fig 4]. Significantly higher level ($p<0.05$) of INOs activity 29.30% ±0.61 and NBT activity 16.10% ±0.14 in test Group-IIa was observed as compared to the control Group-Ia.

The results of probiotic consumption in hypertensive patients are shown in the fig. 5. There was 6.93% ±2.0 decrease in systolic blood pressure level in test Group-IIb on fermented milk product for 30 days. However, no marked difference in systolic blood pressure level in control Group-Ib was observed [Table/Fig 5]), In addition there was enhancement in immune response [Table/Fig 3], [Table/Fig 4] in these subjects as observed by enhanced INOs and NBT activity. The INOs activity and NBT reduction was 13.13% ±0.27 ($p>0.05$) and 4.56% ±0.0.04 ($p>0.05$) more respectively in test Group-IIb as compared to the control group-Ib. Our results show, though insignificant, yet an inverse relationship between the immune activity and level of blood pressure.

Discussion:

In the present study the effect of probiotic consumption in the form of *'Dahi'* and '*lassi'* was studied on immune response, cholesterol level and blood pressure in normal and hypertensive subjects. Results reveal that consumption of these fermented products show hypocholesteremic, immunomodulatory and hypotensive effect. Our observation that probiotic diet can lower the cholesterol level is in agreement with other experimental [3],[31] and clinical studies [14]. Ashar et al. [19] reported hypocholesterolemic effect of probiotic diet in humans and showed total cholesterol reduction to an extent of 12-21% by feeding on acidophilus milk. The lactic fermenters of yogurt reduce the levels of total cholesterol, HDL-C and LDL-C, in a well-balanced way by assimilation of cholesterol by bacteria in youghurt (*in vitro*) [12]. Similar findings were earlier observed by us in experimental animals [18]. The effect of probiotic intake on cholesterol level on human subjects carried by Fabian and Elmadfa [24] was quite like our study which supported consumption of fermented milk leads to decrease in cholesterolemia. In our study, reduction in total serum cholesterol and small difference in HDL-C contents in the humans fed on diet containing lactic acid bacteria was observed. Similar results in humans were also reported by Keim et al. [21]. However, variable effect of different probiotic microorganisms on serum cholesterol has been given in a review by Shah, 2007 [32]. In a study performed by Hashimoto et al. [23], a diet containing *L. casei* TMC 0409 was found to raise the concentration of HDL-C in the blood. Besides, Fukushima and Nakao [22] and Simons et al. 2006 indicated no significant difference in the HDL-C content or cholesterol level corresponding to supplement of probiotics, including *Lactobacillus* and *Streptococcus*, in lipid-rich and cholesterol-rich diets.

In our results the higher values of INOs and NBT in test groups than the control groups show stimulation of systemic immunity. This supports the earlier findings that the probiotic diet might modulate and stimulate immune response [2],[4],[7],[8]. Fermented low fat milk product '*Lassi*' and '*Dahi*' feeding reduced the cholesterol level and increase immune activity in humans. A similar correlation between immune response and cholesteremia has been earlier observed in our previous study [10]. Moreover, Matsuzuki and Chin 2000 [26] reported the oral administration of *Lactobacillus casei* strain *Shirota* (LcS) has been found to enhance innate immunity by stimulating the activity of splenic NK cells. However Christensen et al. 2006 [27] could not find any effect of probiotic supplemented diet on immune response of young healthy adults, even with high doses of *B. animalis ssp. lactis* BB-12 and *L. paracasei ssp. paracasei* CRL-43. But Gill et al. [28] observed enhancement in cellular immunity in the elderly on *B. lactis* as probiotic dietary supplement.

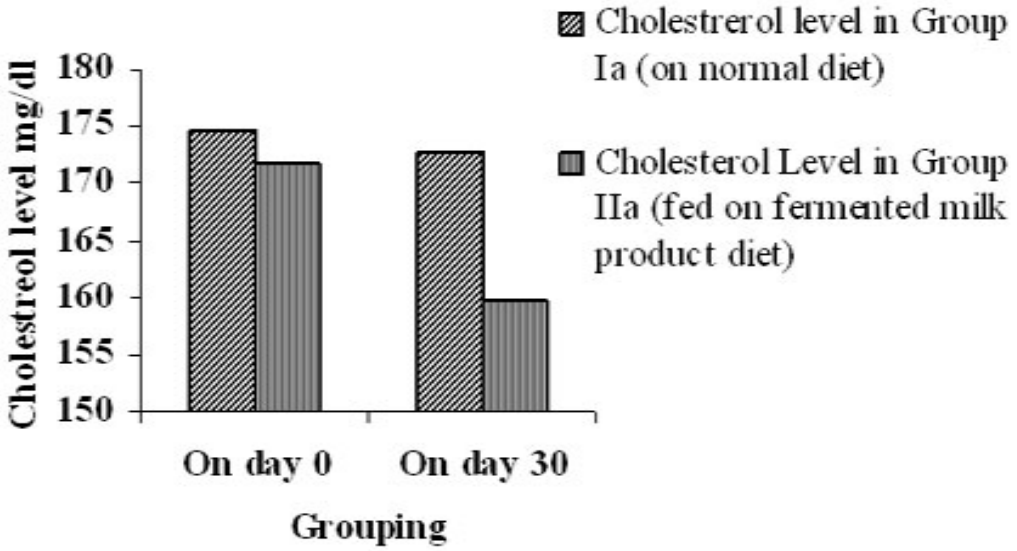
In our results the test groups show enhancement in immune response with simultaneous decrease in blood pressure. Our findings corroborate the earlier report by Kawase et al [15] who stated decrease in systolic blood pressure by fermented milk products.

The role of fermented food in lowering of risk for cardiovascular diseases has been shown earlier by Frick et al. [17] and reduction in serum cholesterol level was also associated with

yogurt intake in humans [7],[21],[29] demonstrated that intestinal lactic acid bacteria, such as *L. acidophilus*, caused bile salts to de-conjugate and co-precipitate with cholesterol under anaerobic conditions. In addition to this, Nielson and Gilliland [20] also showed the cholesterol-reducing activity of *Lactobacillus casei*. On the other hand Schultz et al. investigated stimulation of cellular immune system by *Lb.* GG of healthy volunteers and offered a promising tool to investigate systemic immunomodulation due to oral administration of probiotic microorganisms [29]. Kiyoshi et al. [9] reviewed multifunctional roles of macrophages on atherosclerosis in human and experimental animals which showed correlation between cholesterol level and immune response. In our study an inverse correlation between immune status, cholesteremia and blood pressure has been observed and the same is maintained even by the modulation by probiotics. This is supported by our previous study in mice and also by the study of Kiyoshi et al. [9].

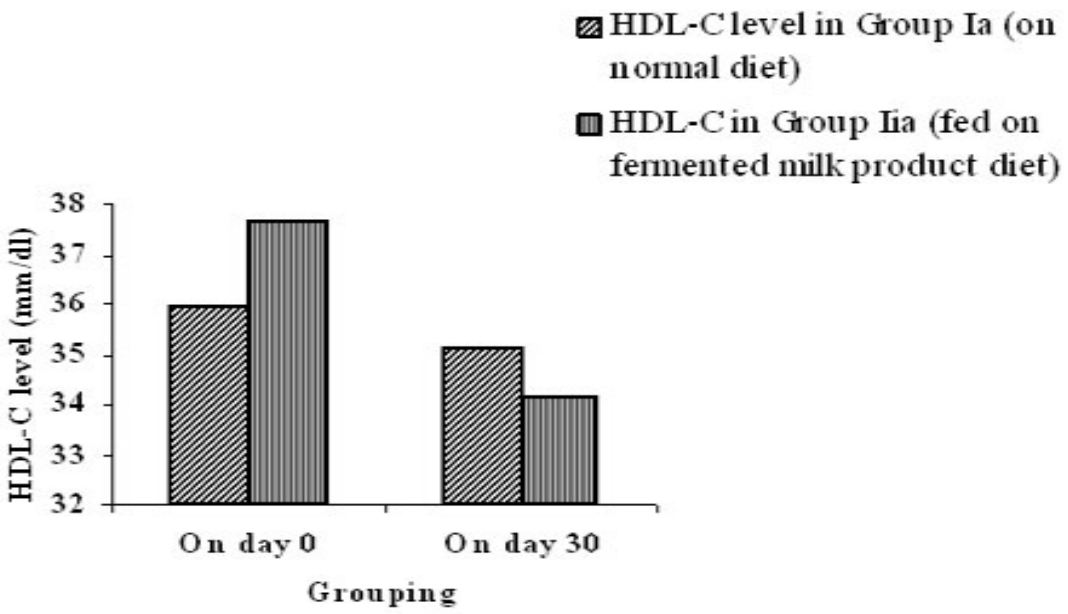
Significance of the study and conclusion:
The available literature reveals that cholesterol and hypertension both causes of atherosclerosis can be modified by the intake of probiotic diet. Diet therapy is cost effective to drug therapy. Moreover the unwanted side effects of the drug can also be minimized. Results reveal that dietary intake of fermented milk product '*Dahi*' and '*Lassi'* by humans' results in decrease in total serum cholesterol and systolic blood pressure and on the other hand significantly increase INOs and NBT activity of macrophages, which show the inverse and indirect relationship of cholesterol and blood pressure with immune activity. Our, this study clearly projects the usefulness of the probiotic containing diet as safe alternative immunotherapeutic agent and functional food for the persons suffering from hypercholesterolemia, hypertension and immune disorder. Not only this, the immune system of body further controls many disorders like diabetes, diarrhea, cancer etc. hence probiotics can improve the overall health of the consumer.

Table/Fig 1



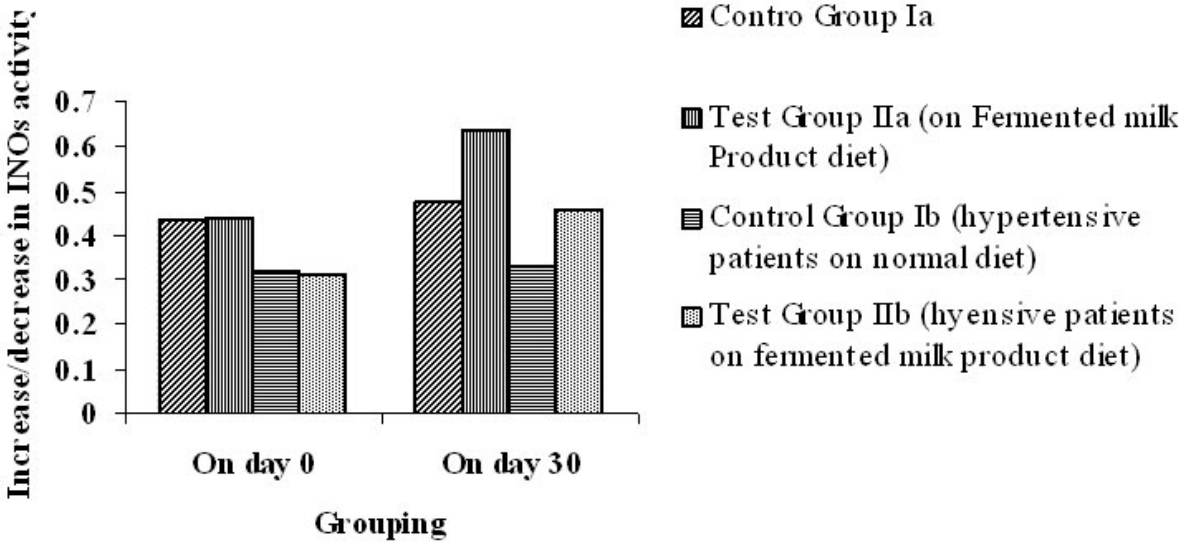
Effect of *'Dahi'* and *'Lassi'* consumption on serum cholesterol level in humans.

Table/Fig 2



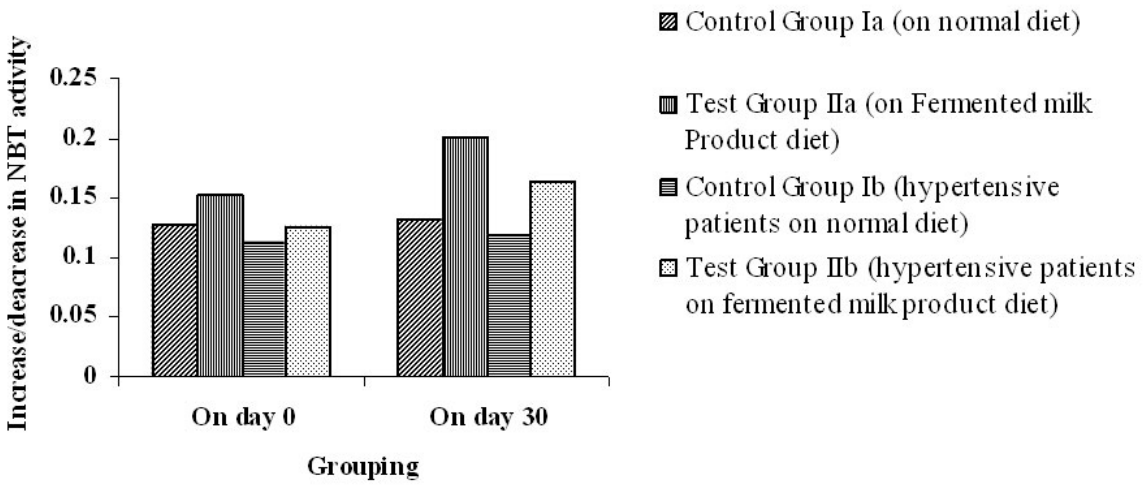
Effect of *'Dahi'* and *'Lassi'* consumption on serum HDL-C level (mg/dl) in human.

Table/Fig 3



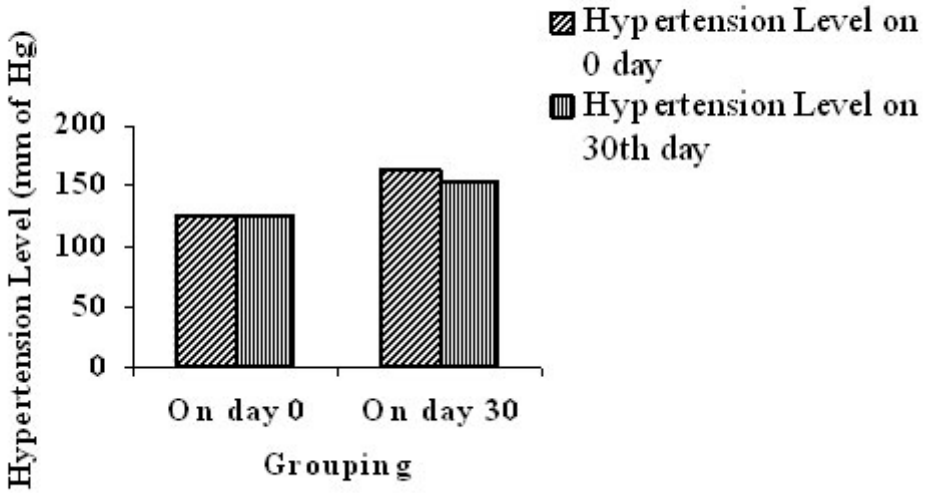
Effect of *'Dahi'* and *'Lassi'* consumption diet on INOs activity in healthy and Hypertensive human subjects.

Table/Fig 4



Effect of *'Dahi'* and *'Lassi'* consumption on NBT reduction in healthy and hypertension human subjects.

Table/Fig 5



Effect of *'Dahi'* and *'Lassi'* consumption on systolic blood pressure level in humans.

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