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Original article

Does Probiotic Therapy Have Effect on Serum Calcium and Cholesterol Levels in Demyelinated Hippocampus?

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Abstract

Background: Recently, the use of probiotics in preventing and treating the immune system diseases through changes in blood factors has attracted the attention of researchers. Therefore, the aim of this study was to evaluate the effect of *Lactobacillus plantarum* and *Bifidobacterium B94* on changes of blood factors, influencing the autoimmune system diseases.
Materials and Methods: The rats used in this study were divided into four groups (n=10 each), including control (saline), damage with Ethidium bromide (EB), *L. plantarum* and *Bifidobacterium B94* treatment groups. In damage and treatment groups, a single dose of 3µL EB was directly injected into hippocampus of rats for inducing demyelization. Also, in control group, the same amount of saline was used. Then 2×10^8 probiotic bacteria were administered by gavage for 28 days. Then serum calcium and cholesterol levels were measured. Data were analyzed by one-way ANOVA and Tukey post-hoc tests ($p\leq .05$).
Results: The results showed that level of blood serum calcium increased insignificantly in the *L. plantarum* and *Bifidobacterium B94* treatment groups compared to control group. Also, the level of blood serum cholesterol decreased insignificantly in both treatment groups compared to control group.
Conclusion: Probiotics are used for preventing and treating some of the common autoimmune diseases such as MS. Previous studies showed that probiotics affects some of the blood parameters such as calcium and cholesterol while decrease or increase in these parameters is effective in the improvement of MS. Although no significant finding has been obtained in some of these studies, they have almost confirmed the recommendation of probiotic consumption.

Keywords: Probiotic, Demyelination, Ethidium bromide, Lactobacillus plantarum, Bifidobacterium B94

1. Background

Multiple sclerosis (MS) is a chronic inflammatory demyelinating autoimmune disease associated with human central nervous system (CNS), which is manifested with varying clinical course, pathology, and inflammatory patterns (1-2) .

Among the various CNS sites involved in the course of MS, the hippocampus is particularly vulnerable to the detrimental effects of neuro inflammation (3-5).

Probiotics are defined as live microorganisms which confer a health benefit on the host when administered in adequate amounts (6). Several previous clinical studies indicated that probiotics may represent a capable preventive and therapeutic strategy for chronic inflammatory diseases (7-8) . Furthermore, it has been reported that probiotics have also great influence on blood parameters (9-10) . The effects of blood parameters and dietary supplements on the course of MS have attracted the attention of scientists. In many studies, the blood parameters and also nutritional factors including iron (11), calcium (12), magnesium (13), vitamin D (14), milk proteins , gluten , antioxidants (uric acid, Vitamins A, C and E, lipoic acid), polyphenols, Ginkgo biloba extracts, and curcumin have been evaluated for their effect on MS disease progression. (15).

Previous in vivo studies indicated that probiotics are effective in improving lipid profiles, including the reduction in serum cholesterol, LDL cholesterol, and triglycerides or increase in HDL cholesterol (10, 16)**.** In another study, it has been reported that consumption of nutritional factors increases the cholesterol. Cholesterol of serum and stimulated immunological responses increase inflammatory diseases such as MS (17).

Few experimental studies indicated that consumption of probiotics results in maintaining the serum calcium in a normal range in animal models (18-19). It was also proposed that calcium is essential for the development, structure and stability of myelin (20-21).

This study was performed to investigate the effects of *L. plantarum* and *Bifidobacterium B94* on calcium and cholesterol parameters on the risk of developing MS in rat models.

2. Objectives

As regards that treatment in MS is very important and blood factor changes can be effective in MS and also probiotics effect on blood factors, this study performed with aims evaluation.
The effect of lactobacillus plantarum and bifidobacteriumB94 on changes of blood factors that can influence on the autoimmune system diseases.

3. Materials and Methods

3.1. Animals

Forty male Wistar rats weighing from 200-250 g were obtained from Pasteur Institute of Iran (IPI). They were

maintained under controlled light conditions (12h light/dark cycle), room temperature (32 ± 2°C), 50-60% humidity. All experimental techniques involving animals were performed in Alborz University of Medical Science.

3.2. Microinjection of ethidium bromide and experimental design

Animals were anaesthetized with chloral hydrate (80 $mg.kg^{-1}$, i.p.), and the rats were fixed by stereotaxic instrument in the skull-flat position. Demyelination was induced unilaterally by direct single injection of 3 µL of 0.01% ethidium bromide (EB) in sterile 0.9% saline with a Hamilton syringe at the rate of 1 $\mu L.min^{-1}$ into the right dentate gyrus of hippocampal formation, using appropriate stereotaxic coordinates (AP =-2.8; ML =+1.8; DV =+2.5) [22]. The animals were randomly divided into 4 groups (n=10 each), the control negative group received intra-hippocampal injections of saline without EB, the control positive group received saline as solvent of the EB with no treatment (damage group), and the experimental groups received *L. plantarum* and *Bifidobacterium B94* by oral gavage once per day for 28 days after EB injection. Groups treated by gavage were compared to rats receiving no treatment.

3.3. Preparation and cultivation of probiotics bacteria

Two probiotic strains of *L. plantarum* and *Bifidobacterium B94* were purchased from DSM. Both probiotics were grown anaerobically on Mann–Rogosa–Sharpe (MRS) broth at 37°C for 48 h (23). Probiotic cultures were centrifuged at 3000 rpm for 10 min, the supernatants were discarded, and the probiotic content vials were washed by PBS solution, then vials were re-centrifuged. Probiotic content vials were diluted in normal saline and then were prepared based on 0.5 McFarland standards (1.5×10^8 $cfu.ml^{-1}$). This dilution was used for 28 days.

3.4. Measurement of calcium, and cholesterol serum levels

After day 28, rats were randomly selected from each group, and 3 mL of blood samples were collected from heart. The collected blood samples were centrifuged at 3000 rpm for 10 min. The blood serum was transferred into all aseptic treated vials by Pastuer pipette and stored at –20˚C for further analysis. The serum cholesterol and calcium were determined colorimetrically using CHOD-PAP and Arsenazo/Endpoint methods, respectively by pishtazteb kit.

3.5. Statistical analysis

Data were scrutinized using one-way analysis of variance (ANOVA) followed by the Tukey post hoc. The results are expressed as mean ± SEM. The statistical limit for accepting significance was $p < .05$.

4. Results

4.1. Effect of probiotic on serum calcium level

The effect of probiotics on serum calcium total concentrations was shown in experimental groups (Figure 1). It was shown that the amount of serum calcium in the treatment groups was not significantly increased by probiotics (by *L. plantarum*, 2.6±0.05 and *by Bifidobacterium B94*, 2.5±0.03) compared to control and damage by EB groups. The amount of blood serum calcium in EB group was 2.4±0.02, and in control group was 2.45±0.04.

4.2. Effect of probiotic on serum cholesterol level

The effect of probiotics on serum cholesterol total concentrations was shown in experimental groups (Figure 2). It was shown that blood serum cholesterol in the treatment groups was decreased insignificantly by probiotics (by *L. plantarum*, 82±3 and by *Bifidobacterium* B94, 81±4) compared to control and damage by EB groups. The amount of serum cholesterol in control group was 84±3, and in EB group was 85±2.

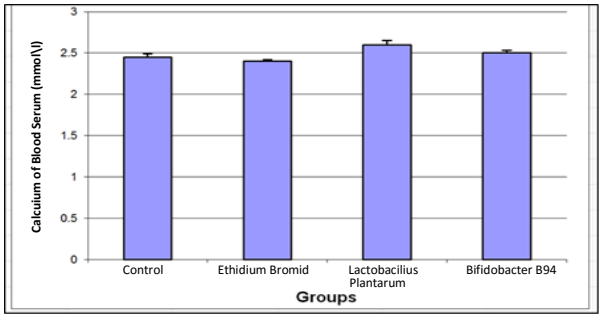

Figure 1. Effect of administering *lactobacillus plantarum* and *bifidobacterium* B94 probiotics on the amount of calcium in blood serum in control, damage with EB, and treatment groups.

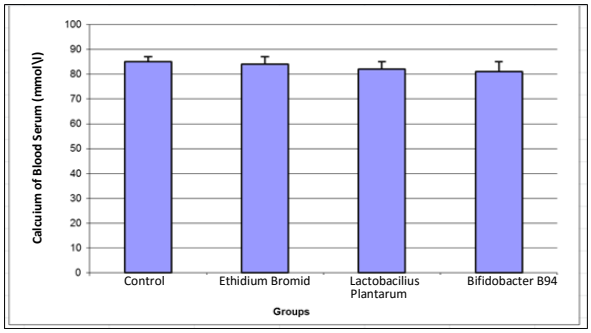

Figure 2. Effect of administering lactobacillus plantarum on serum cholesterol in control, damage with EB, and treatment groups.

5. Discussion

Multiple sclerosis is an inflammatory immune mediated demyelinating disorder in which remyelination failure contributes to persistent disability (1). A recent systematic review described high effect of blood parameters on chronic inflammatory diseases such as MS (15). Also, several studies have indicated that probiotics have great influence on blood parameters (9-11, 16, 24).

In this experiment, the effects of two probiotic bacteria of *L. plantarum* and *Bifidobacterium B94* were investigated on serum cholesterol and calcium parameters concentrations, and consequently, on MS disease in rat models.

It has been demonstrated that various probiotic species decrease the serum cholesterol levels in human (25), experimental animals (26) or farm animals (27-28). This hypocholesterolemic effect has been suggested to be caused by more than one mechanism. The recent studies have reported that probiotics affect gene expression of a carrier protein called Niemann-Pick C1-like 1 (NPC1L1) which is responsible for cholesterol absorption. Reduction or inhibition from the expression levels of this protein leads to a decrease in plasma cholesterol levels [29]. Fazeli et al. reported that daily gavage of *L. plantarum* A7 decreases serum cholesterol total concentrations in rats (30). In another study, *L. plantarum* PHO4 reduced total cholesterol level in hypercholesterolemic

mice (31). Taranto et al. demonstrated that during a 7-day study, *L. reuteri* reduced the total concentration of serum cholesterol in mice (32). Bernini et al. reported the potential effects of *B. lactis* HN019 on reducing blood lipids (33). A clinical trial showed that LDL-C decreased after supplementation with specific probiotics of *L. acidophilus and B. lactis* in Type 2 diabetic people (34). Another study showed cholesterol-lowering effects of a probiotic formulation containing three *Bifidobacterium* strains in hyperlipidemic children (35) while in other studies no effects was shown; for instance, in a study by Ivey et al., the probiotic strains of *L. acidophilus* La5 and *B. animalis* subsp. *Lactis* Bb12 did not significantly alter serum lipid concentrations (p> .05) (36). In a study conducted by Greany et al., the results did not support the beneficial effect of *L. acidophilus* DDS-1 strains and *B. longum* UABL-14 strains on plasma lipids in normocholesterolemic young women and men (37). These inconsistent results can be attributed to different strains and doses of probiotics, delivery matrix, study duration, and study heterogeneous population.

Our data are in agreement with Ivey et al. and Greany et al. studies in which *L. plantarum* and *Bifidobacterium B94* did not significantly alter serum cholesterol concentrations (p > .05) in rat models.

Cholesterol is rate-limiting for myelin biogenesis in the developing CNS; however, it is unclear whether cholesterol insufficiency contributes to remyelination failure in MS.

Earlier studies have demonstrated that cholesterol availability is a prerequisite for myelination (38-40). However, the insignificant effects of lowering plasma cholesterol on MS disease amelioration suggested by these data are presently uncertain and deserve further studies.

Calcium is the most abundant and one of the most important minerals in the human body. Approximately 99% of body calcium is found in bones (41). Few studies showed that consumption of probiotics results in maintaining the serum calcium levels in the normal range (18-19) . It was also proposed that calcium is essential for the myelin sheath repair and recovery process in neurodegenerative diseases like MS (21).

Previous studies showed that some probiotics could increase serum calcium levels by improving calcium bioavailability (42), solubility, and absorption from intestinal tract (19). Gilman et al. (2006) reported that short chained fatty acids and the other products produced by the bacteria decrease the pH of intestines microenvironment upon which calcium solubility increases, and this may be related to increased calcium absorption (43).

Vinderola et al. (2007) reported that supernatant from milk fermented by *L. helveticus* R389 enhanced the expression of calcium channels, indicating an improved capacity for dietary Ca^{+2} uptake (44).

In a study conducted by the Narva et al., it was reported that fermentation of milk with *L. helveticus* had a positive acute effect on calcium metabolism in postmenopausal women, indicating the increase in serum calcium levels (45).

In a study by Asemi et al., the consumption of probiotic yogurt in comparison with conventional yogurt among the pregnant women resulted in the maintenance of serum calcium levels (p = .01) (46).

Gohel et al. study reported an increase in serum calcium level caused by well-documented probiotic of *L. helveticus* MTCC 5463 when administered to geriatrics (18).

In this study, serum calcium levels increased in the experimental groups but not significantly compared to control groups. The present findings are in agreement with the results reported by Kunavue and Lien who stated that calcium was not different between the control and probiotic-treated groups (11) and by Hashemzadeh et al. (2013) who reported results in Broiler Chicks (47). High correlations have been found between serum calcium levels and MS amelioration (21, 48). In a clinical study conducted by P. Goldberg, it was found that MS patients had less MS exacerbations when taking calcium, magnesium, and vitamin D over a two-year period of time. He reported that these nutrients strengthened the myelin sheath to resist damage caused by multiple sclerosis (49).

6. Conclusion

Taken together, our data showed that consumption of *L. plantarum* and *Bifidobacterium B94* for 4 weeks did not have significant effect on serum cholesterol concentrations, and no significant differences was observed in total amount of calcium. This study highlights the importance of probiotics consumption in order to alter serum cholesterol and calcium concentrations. This finding might have implications for the management of demyelinating diseases, but further studies are required to determine consumption of probiotics feasibility for patients.

Conflicts of interest

The authors declare they have no conflict of interests.

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Authors' Contributions

In the study, the 8 authors have worked on cases referred: Surgery and demyelination induction of rats by EB was performed by Mahdi Goudarzvand, Somayeh Soleymanzadeh Moghadam and Mehdi Kamal Zare. Activities related to probiotics were performed by Zohreh Khodaii and zeinab Fagheei Aghmiyuni. Statistical analysis was performed by Sara Fathi Zadeh. Manuscript was written by Somayeh Soleymanzadeh Moghadam and Nazanin mohammad. Manuscript was edited by Mahdi Goudarzvand and Ali majidpour.

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