



Effects of Probiotics, Prebiotics and Symbiotics on Serum Cholesterol Levels

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Abstract

Objective: The aim of this study is to analyze the impact of probiotics, prebiotics, symbiotics on lipid metabolism in hypercholesterolemia.

Materials and Methods: A total of 30 Wistar rats were divided into 5 groups and fed the appropriate diets. Group 1 was given only normal rat chow. Group 2 were given cholesterol rich diet, group 3 were given cholesterol rich diet and probiotics, group 4 were given cholesterol rich diet and prebiotics, group 5 were given cholesterol rich diet and symbiotics for 21 days.

Results: Mean values of AST in groups were 123 ± 1.1 U/l, 124 ± 1.18 U/ml, 135 ± 1 U/ml, 136 ± 0.89 , 143 ± 0.78 U/ml. Mean values of ALT in groups were 72 ± 0.89 U/l, 73 ± 1 , 74 ± 0.78 U/ml, 86 ± 1.1 , 95 ± 0.63 U/ml. Mean values of total cholesterol in groups were 51.7 ± 0.91 mg/dl, 53.5 ± 1 mg/dl, 45.8 ± 1 mg/dl, 45.0 ± 0.89 mg/dl, 44.4 ± 1 mg/dl. Mean values of LDL cholesterol in groups were 17.2 ± 0.77 mg/dl, 25.1 ± 0.63 mg/dl, 14.0 ± 0.63 mg/dl, 11.9 ± 0.89 mg/dl, 10.9 ± 0.89 mg/dl. Mean values of HDL cholesterol in groups were 17.8 ± 0.89 mg/dl, 11.4 ± 0.77 mg/dl, 15.9 ± 0.91 mg/dl, 17.0 ± 0.89 mg/dl, 17.5 ± 0.91 mg/dl. Mean values of triglycerids in groups were 83.6 ± 0.63 mg/dl, 85.0 ± 0.63 mg/dl, 79.6 ± 0.89 mg/dl, 80.6 ± 0.77 mg/dl, 80.1 ± 0.77 mg/dl.

Conclusion: Probiotics and prebiotics improve lipid metabolism but alter AST and ALT values in rats, especially when they are used in combination.

Keywords: Glutathion; Diabetes mellitus; Complications

Introduction

Probiotics are bacteria's that improve microbial balance in the mucose membranes and digestive system so that can help host's mucosal and systemic immunity. Prebiotics are non digestible carbohydrates and strengthen the effects of probiotic microorganism by increasing their colonisation. Use of probiotics and prebiotics together is defined as symbiotic. First, in 1974 cholesterol lowering effect of lactobacilli fermented milk in humans has been stated [1]. Certain species of Lactobacillus and Bifidobacterium reduced blood cholesterol levels [2]. Femia showed that the use of probiotics and prebiotics together, provided additional but not synergistic effect [3]. The most widely used probiotic bacterias are lactic acid bacteria belonging to the genus Lactobacillus species (spp), Bifidobacterium spp, Enterococcus spp, Streptococcus spp and other bacterias are Basillus spp, Saccharomyces spp and Propionbacterum species [4]. There is no known effective dose for probiotics but 10^9 Colony Forming Unit (CFU)/day is recommended. Probiotics must be alive and sufficient number in the intestine to demonstrate an effect [5]. Kaushik [6], Paik [7] and Lim [8] showed that probiotics lowered cholesterol and triglycerid levels significantly in animal trials. Anderson [9] and Kondo [10] demonstrated beneficial effects of probiotics on cholesterol metabolism in human trials. Reimer and Russel [11], Liong and Shah [12] showed that only prebiotics lower the cholesterol, Kiebling [13], Gibson and Roberfroid [14] showed the usefulness of symbiotics in hypercholesterolemia. In our study, we aimed to determine the effects of probiotics, prebiotics and symbiotics on serum cholesterol levels in cholesterol rich fed rats.

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Table 1: Results.

	Group I control	Group II Cholesterol	Group III Cholesterol+ Probiotic	Group IV Cholesterol+ Prebiotic	Group V Cholesterol+ Symbiotic
AST U/l	123±1.1	124±1.18	135±1	136±0.89	143±0.78
ALT U/l	72±0.89	73±1	74±0.78	86±1.1	95±0.63
Total kolesterol mg/dl	51.7±0.91	53.5±1	45.8±1	45.0±1	44.4±1
LDL kolesterol mg/dl	17.2±0.77	25.1±0.63	14.0±0.63	11.9±0.89	10.9±0.89
HDL kolesterol mg/dl	17.8±0.89	11.4±0.67	15.9±0.91	17.0±0.89	17.5±0.91
Trigliserid mg/dl	83.6±0.63	85.0±0.63	79.6±0.89	80.6±0.77	80.1±0.77

Materials and Methods

Ministry of Health Animal Experiments Ethic Committee approved the study. National research council's guide for or use of laboratory animals was followed. In this study, 30 Wistar rats with average weight of 250 grams were used. Rats were divided 5 into groups. All groups were fed with appropriate nutrients for 21 days under appropriate conditions in the experimental animal's laboratory. At the end of 21 days following the blood sampling the rats were anesthetised by ether inhalation to induce long term anesthesia analgesia. Ketamine 10 mg/kg (0.2) dose was injected in to quadriceps muscle. Then they were scarified by creating pneumothorax.

The groups are;

1. Group 1: Control group (n=6). They were fed with regular rat foot.
2. Group 2: Cholesterol rich group (n=6). They were fed with cholesterol rich addition to regular rat foot.
3. Group 3: Cholesterol rich + probiotic group (n=6). They were fed with cholesterol rich addition to regular rat foot and *P. Jensenii* BDP11 (probiotic).
4. Group 4: Cholesterol rich + prebiotic group (n=6). They were fed with cholesterol rich addition to regular rat foot and 1,4-dihydroxy-2-naphthoic acid (DHNA) (prebiotic).
5. Group 5: Cholesterol rich + symbiotic group (n=6). They were fed with cholesterol rich addition to regular rat foot and *P. Jensenii* BDP11 and 1,4-dihydroxy-2-naphthoic acid (DHNA) (symbiotic).

As probiotic, *Propionibacterium jensenii* BDP11 strain which was isolated in Turkey Scientific Technical Research Council (TUBITAK) 107T486 coded identifying Propionic Acid bacteria species found in Turkey's traditional cheese studying probiotics and starter features named project was incubated at 10⁹ CFU/ml concentration in anaerobic media at 30° and the cultures obtained were used as rat fed. The strain was produced from Balıkesir Manyas tongue chese. Hundred mg/ml cholesterol strains was incubated in YEL food medium under anaerobic conditions for 4 days and at the end of incubation period the cell pellets were obtained by centrifugation at 5000 rpm for 15 minutes. Pellets were washed twice with saline solution and 2 ml of physiological saline was resuspended. One ml of this bacterial suspension was given to groups 3 and 5 rats for 21 days. DHNA, stimulating propionibacterium reproduction was used as prebiotic. One percent cholesterol (Sigma C8503) was added to normal rat food for cholesterol rich food. Total cholesterol, LDL cholesterol, HDL cholesterol and triglycerids (TG) values were analyzed in Siemens ADVIA 240 JAPAN auto analyzer by colorimetric method, AST and ALT were analyzed by enzymatic method.

Statistical method

Statistical analysis was performed by using SPSS for Windows 17.0 program. Statistics were performed with One Way ANOVA test. Post Hoc. Tukey test was used to assess differences between groups.

Results

AST values; There was no significant difference between control and cholesterol groups (p=0.16). In probiotic, prebiotic, symbiotic groups there were a significant increase in AST when compared to control and cholesterol groups (p< 0.001). The difference between probiotics and symbiotics groups and between prebiotics and symbiotics groups was significant (p< 0.001). ALT values; There was no significant difference between control and cholesterol groups (p=0.097). In probiotic, prebiotic, symbiotic groups in comparison to control group in prebiotic, symbiotic groups in comparison to cholesterol group increases were significant (p< 0.005). Increases among probiotic, prebiotic and symbiotic groups were significant (p<0.001). Total cholesterol values; cholesterol in the control group increased significantly (p=0.009). In probiotic, prebiotic, symbiotic groups cholesterol decreased significantly when compared cholesterol group (p<0.001). Decrease in symbiotic group was significant in comparison to probiotic group (p=0.036) whereas not significant compared to prebiotic group (p=0.298). LDL cholesterol values; values were cholesterol significantly higher in cholesterol group when compared to control group (p<0.001). In probiotic, prebiotic, symbiotic groups LDL cholesterol decreased significantly in comparison to control and cholesterol groups (p<0.001). Difference between probiotic, prebiotic, symbiotic groups were significant (p<0.001) and the maximum difference was observed in symbiotic group (p<0.001). HDL cholesterol values; decreased significantly in cholesterol group, compared to control group (p<0.001). In probiotic, prebiotic, symbiotic groups HDL cholesterol increased significantly with respect to cholesterol group (p<0.001). However among probiotic, prebiotic, symbiotic groups there was not a significant difference (p=0.06). TG values; increased in cholesterol group with respect to control group (p<0.001). In probiotic, prebiotic, symbiotic groups TG decreased with respect to control and cholesterol groups (p<0.001). This decrease was not significant among probiotic, prebiotic, symbiotic groups (p=0.28) (Table 1). When cholesterol group was compared with other groups, total cholesterol value decreased 14% in probiotic group, 16% in prebiotic group, 17% in symbiotic group (p<0.005). When cholesterol group was compared with control group, LDL cholesterol value increased 46% (p<0.005). When cholesterol group was compared with other groups, LDL cholesterol value decreased 44% in probiotic group (p<0.005), 52.6% in prebiotic group (p<0.005), 56.6% in symbiotic group (p<0.005). When cholesterol group compared with control group, HDL cholesterol values decreased 36% (p<0.005). HDL cholesterol value increased 39.5% in probiotic group (p<0.005), 49.1% in prebiotic

group ($p < 0.005$), 53.5 % in symbiotic group ($p < 0.005$).

Discussion

In order to increase the effect of probiotics and prebiotics, symbiotic use became a current issue in many studies [15]. Nguyen applied *Lactobacillus plantarum* PHO4 to hypercholesterolemic rats for 14 days [16]. Park gave cholesterol rich food to rats for 21 days to induce hypercholesterolemia [17]. In our study, when the development of hypercholesterolemia is evaluated, the increase in total cholesterol, LDL cholesterol levels and decrease in HDL cholesterol levels were significant in cholesterol group, with respect to control group ($p = 0.009$), ($p < 0.001$). Considering these findings, planned 21 days of study period planned for this study can be stated to be sufficient to provide the development of hypercholesterolemia. In our study in groups 2,3,4,5 elevated ALT and AST were found but it was not significant among control and cholesterol groups ($p = 0.09$). It was significant between control group and probiotic, prebiotic, symbiotic groups ($p < 0.001$). When cholesterol group was compared with other groups, a decrease of 14% in probiotic group ($p < 0.001$), 16% in prebiotic group ($p < 0.001$), and 17% ($p < 0.001$) in symbiotic group was observed. Our data are compatible some of the trials (19,20). LDL cholesterol levels showed significant difference between groups ($p < 0.001$). Total cholesterol was 45% higher in cholesterol group, with respect to control group ($p < 0.001$). When cholesterol group was compared with other groups LDL cholesterol value was 44% lower in probiotic group ($p < 0.001$), 52.6% lower in prebiotic group ($p < 0.001$) and 56.6% lower in symbiotic group ($p < 0.001$). HDL cholesterol levels in groups 2,3,4,5 were not increased. HDL cholesterol value in cholesterol group was 36% lower when compared to control group. When cholesterol group was compared with other groups, HDL cholesterol value increased 39.4% in probiotic group ($p < 0.001$), 49.1% in prebiotic group ($p < 0.001$) and 53.5% in symbiotic group ($p < 0.001$). In Paik's animal trial while a significant decrease in LDL cholesterol was observed, decrease in total cholesterol was not significant. TG and HDL cholesterol levels were not affected (7). Xiao, observed a significant reduction in total cholesterol, LDL cholesterol, TG levels and 14.5% significant increase in HDL cholesterol levels in humans [18]. In our study, between probiotic, prebiotic, symbiotic groups and control, cholesterol groups TG values were significantly less ($p < 0.001$). However the differences between probiotic, prebiotic, symbiotic groups were not significant ($p = 0.06$). Preliminary data from two pilot non-randomised studies suggest that probiotics may be well tolerated, may improve conventional liver function tests [19]. This is the first study to use *P. jensenii* BDP11 and DHNA. Increase in AST and ALT values may be due to difference in their profile of effect.

Conclusion

In this study probiotics, prebiotics and symbiotics significantly reduced total cholesterol, LDL cholesterol and Tg levels in hypercholesterolemia induced rats. This effect is greater in LDL cholesterol value with symbiotics. In rats fed with normal food, there is no enhancer effect on HDL cholesterol but they prevent HDL cholesterol reduction fed with high cholesterol diet. However results have no superiority to each another. Despite these positive effects, increase in AST and ALT values is negative results of this study. This result may be due to *P. jensenii* BDP11 strain and DHNA which were not used previously.

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