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The effect of yogurts containing *Gundelia tournefortii* L. extract, kefir and probiotics on the serum calcium, phosphorus and lipid profile of rat

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Original Article

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Abstract:

Background and aims: The beneficial effects of probiotics, especially yogurt, have drawn attention, but in the last few years, probiotic yogurts have received great attention due to their significant effects on health. Plants, including artichoke (*Gundelia tournefortii* L.), have many beneficial effects. In this study, the effect of yogurts containing *G. tournefortii* extract and kefir on lipid, lipoprotein pattern, calcium and phosphorus was compared with that of probiotic yogurts obtained from two microorganisms, *Lactobacillus acidophilus* and *Bifidobacterium bifidum*.

Methods: 12 batches of probiotic yogurts were produced by Tamime method, along with kefir and *G. tournefortii* extract. The yogurts along with a high-fat diet were fed to 80 adult male Wistar rats for seven days. At the end of study, the serum levels of lipid, lipoproteins, calcium and phosphorous were measured using standard methods.

Results: The effect of yogurt containing *G. tournefortii* extract was similar to those of probiotic yogurts in lowering serum cholesterol. Although yogurts containing *G. tournefortii* extract and probiotic yogurts pronouncedly reduced HDL-C, VLDL-C, LDL-C and triglycerides, the reduction was not statistically significant. There were no

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significant differences between the mean concentrations of phosphorous in different groups.

Conclusion: Yogurts containing *G. tournefortii* extract have similar effects on the reduction of high-fat diet-induced hypercholesterolemia to those of probiotic yogurts containing *L. acidophilus* and *B. bifidum*. The usage of *G. tournefortii* extract 0.9%, kefir and 0.6 g *L. acidophilus* increases the calcium level compared to normal diet, but the other *G. tournefortii* extracts and probiotic yogurts do not affect calcium levels.

Keywords: Gundelia tournefortii L., Kefir, Lipid, Probiotic, Yogurt

INTRODUCTION

In recent decades, the production process of probiotic products, especially probiotic yogurt, has become more popular (1).

In numerous studies, very valuable properties have been reported for probiotics, including facilitating lactose digestion, contributing to acquiring resistance to intestinal pathogens, and producing inhibitory effect on some cancers. Therefore, the production and supply of probiotic products to the consumer market is nutritionally useful for the community (2, 3, 4).

L. acidophilus and *B. bifidum* are normally part of a complex gastrointestinal ecosystem and are traditionally used in the production of fermented dairy products. Due to the beneficial effects of these bacteria on health, they are classified as probiotics. Lowering blood cholesterol is one of the benefits of these bacteria (5,6).

Lactobacillus acidophilus and *Bifidobacterium bifidum* are considered to be the most important probiotic bacteria and have beneficial effects on the health of the host. Probiotics are microorganisms that produce beneficial effects by improving the microbial balance of the body's natural microflora (7).

Kefir is produced from the fermentation of milk with kefir grains and the mother environment prepared from the grains. The product of kefir is obtained from *Lactobacillus kefiranofaciens*, which is abundant in the kefir grain and its center (8).

In different countries, herbal medicines play an important role in the treatment and prevention of various diseases. Desert artichoke (French: *chardonnette*) is a perennial plant of the genus Compisitae. The scientific name of the plant is *Gundelia tournefortii* L. This plant has a variety of minerals and

is used in vegetarian dishes. There are several uses and important substance that is extract by new methods such as, hypolipidemic, sterols and fat acid composition, antioxidant, atherosclerosis, suitable for diabetes(9).

Cardiovascular diseases are the most important cause of death in many countries (10) including Iran. According to current statistics, cardiovascular diseases account for approximately 40% of deaths in the country (3). One of the most important risk factors for these diseases is high cholesterol (11). Given that a 1% reduction in serum cholesterol reduces the risk of coronary heart disease by 2-3% (12), lowering blood cholesterol using treatment regimens or food intervention is one of the ways that can greatly reduce mortality rate from these diseases in Iran (12).

The presence of the probiotic bacterium *Lactobacillus rhamnosus* GG in probiotic yogurt reduces lipid metabolites especially cholesterol in rats, which is a major factor for the comparably lower levels of cholesterol and triglycerides in probiotic yogurt compared to probiotic-free yogurt. The second reason is the pH of probiotic yogurt and the greater activity of probiotic bacteria at the optimal pH for the growth of these bacteria.

The third reason is the properties of probiotics, including their resistance to stomach and intestinal bile acid, tolerance at pH (2-3) and high concentration of bile (0.2-2%) (4), as well as the presence of hydroxyl methyl glutarate in probiotic yogurt, a probiotic that is produced by yogurt starter microorganisms during the fermentation process and inhibits cholesterol synthesis in the body, and thus reduces the amount of cholesterol in the blood (2,4).

Another reason for lowering cholesterol, triglycerides, and lipid metabolites is the activity of hydrolyzing enzymes in probiotic bacteria that conjugate in the enterohepatic circulation and thus affect the production of bile salts that are the end product of cholesterol metabolism (13); as a result, probiotic bacteria reduce lipid metabolites in the body by their hydrolyzing activity (13, 14).

The aim of this study was to compare the effect of one-week consumption of yogurts containing *L. acidophilus* and *B. bifidum* and probiotic kefir and yogurts containing

G. tournefortii hydroalcoholic extract and kefir on the serum lipid profile of high-fat diet-fed rats.

MATERIALS AND METHODS

Study design:

One liter of low-fat sterile milk was cooled down to 20-25 °C, and then kefir grains were added and the resulting mixture was incubated under this temperature for 24 h. After 24 hours, the kefir grains were removed and the produced kefir was left in a refrigerator at 4 °C; then 12 containers were prepared and 1 liter of sterilized low-fat milk was poured into each container.

In addition to the above-mentioned items, *G. tournefortii* hydroalcoholic extract 0.3%, 0.6% and 0.9% was added to yogurts 1, 2 and 3, respectively, 0.1, 0.3 and 0.6 g of lyophilized *L. acidophilus* and 0.6% *G. tournefortii* hydroalcoholic extract to yogurts 4, 5 and 6, respectively, 0.1, 0.3 and 0.6 g of lyophilized *B. bifidum* and 0.6% *G. tournefortii* hydroalcoholic extract to yogurts 7, 8 and 9, respectively, and 0.1, 0.3, and 0.6 g of lyophilized *B. bifidum* and *L. acidophilus* and 0.6% *G. tournefortii* hydroalcoholic extract to yogurts 10, 11 and 12, respectively, in the step of adding the kefir.

In the extract addition step, the milk was stirred thoroughly until the extract was dissolved evenly. Then, all the containers were incubated at 38 °C.

Eighty adult male Wistar rats with an average weight of 180±20 g were selected and kept in 12 h light/12 h dark at 20-25 °C for 15 days to adapt to the environment of the laboratory of animal care center, during which they were fed with water and conventional food. The rats were randomly divided into 16 groups of 5 each. Groups 1-4 were considered as control groups, so that group 1 was given a normal diet, group 2 a high-fat diet, group 3 a high-fat diet and probiotic-free yogurt, and group 4 a normal diet and probiotic-free yogurt.

Groups 5-16 served as treatment groups so that all the 12 groups received seven days of high-fat diet and then, along with a high-fat diet, 10 ml of yogurts 1 to 12 for seven days, respectively. At the completion of the treatment, blood samples were collected from the rats under sterile

conditions and after serum isolation and transfer to the laboratory, calcium was assayed by o-Cresolphthalein *and phosph*orous by colorimetric methods. Total cholesterol and triglyceride levels were measured by enzymatic methods, low-density lipoprotein (LDL-C) by Friedwald's method, and high-density lipoprotein (HDL-C) by the phosphotungstate method. All the parameters were assayed by biochemical kits (Zist Chimi) and spectrophotometer uv-1900 manual (Shimadzu). Very low-density lipoprotein (VLDL-C) levels were estimated by dividing triglyceride levels by 5(VLDL-C = TG/5) (5,13-15).

Statistically analysis:

The Kolmogrov-Smirnov test showed that the data are normally distributed. The results were statistically analyzed using SPSS statistically package (Version 10.0, SPSS Inc.) for ANOVA, Tukey, and t-tests.

RESULTS

The results are presented in Tables 1-6. The ANOVA results showed that there was a statistically significant difference in the mean levels of cholesterol and LDL-C between groups 1 and 2 and other groups (Table 2). Tukey's test results revealed that control group 2 showed a statistically significant difference in cholesterol and LDL-C to some treatment groups. Table 6 shows that there is no statistically significant difference in the mean values of different parameters between control groups 3-5 and other groups. There were no statistically significant differences between the mean serum levels of phosphorous in different groups. Only statistically significant differences were noticed between the mean level of calcium in group 10 in comparison to control group 1 (Table 3).

Table 1: The mean±SD of different parameters in 16 studied groups

Parameter / group	Cholesterol **(mg/dl)**	Triglyceride **(mg/dl)**	HDL **(mg/dl)**	LDL **(mg/dl)**	VLDL **(mg/dl)**	Calcium **(mg/dl)**	phosphorus **(mg/dl)**
1	60.60+5.03	102.80±32.16	32.17±8.14	25.80±3.11	24.20±8.35	18.50±00.60	5.38±00.93
2	81.40+2.70	177.20±52.45	52.45± 6.12	28.20±4.82	32.40±5.41	19.48±1.15	5.56±1.06
3	69.60+7.54	138.40±39.82	39.82±3.65	24.80±3.27	30.80±6.38	20.50±1.48	5.70±1.15
4	56.80+7.66	110.00±48.30	48.29±5.55	25.20±4.87	17.80±3.42	21.08±1.29	5.26±00.29
5	64.20+10.59	89.60±18.21	18.21±5.38	20.40±1.95	23.40±5.03	20.68±2.21	6.48±1.20
6	62.60+7.96	121.60±41.01	41.01±6.76	23.40±4.16	25.00±8.45	19.64±1.83	5.48±00.52
7	62.80+8.04	95.20±18.86	18.86±2.28	21.40±3.78	20.00±3.35	21.78±2.78	5.86±00.97
8	61.00+6.59	140.00±43.73	43.73±4.72	28.80±4.82	27.00±5.61	23.70±3.61	5.46±00.53
9	62.60+7.44	111.20±32.49	32.49±6.98	23.80±4.49	22.20±6.72	22.48±3.14	6.42±00.60
10	65.60+6.65	109.20±25.04	25.04±4.76	23.40±3.58	20.80±5.36	24.42±4.31	5.40±00.32
11	60.60+4.72	92.20±28.22	28.22±4.21	23.80±2.77	18.40±3.78	23.54±3.82	5.72±1.06
12	56.60+5.50	114.80±39.88	39.88 ±7.92	23.60±3.97	22.40±8.32	20.34±1.27	6.12±2.00
13	66.40+10.97	139.40±33.38	33.38±4.09	25.80±3.56	28.60±6.02	20.96±00.86	5.60±00.08
14	68.60+4.83	110.80±36.50	36.50±0.64	23.00±5.43	23.20±7.05	21.04±3.15	6.30±1.53
15	63.80+10.33	114.20±49.25	49.25±7.96	24.40±4.16	22.00±10.07	24.02±3.76	5.28±00.61
16	60.20+4.44	117.00±39.34	39.34±4.45	22.80±2.39	23.20±4.66	21.30±2.44	6.94±1.58

Table 2: Presence or absence of significant differences between the mean levels of different parameters in different groups according to ANOVA test

Cholesterol	Triglyceride	LDL	HDL	VLDL	Calcium	Phosphorus
0.000	0.064	0.069	0.120	0.027	0.016	0.416

Table 3: Presence or absence of significant differences between the mean levels of Cholesterol, triglyceride and calcium in control group compared to different groups

Group / Parameter	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Cholesterol	0.003	0.843	0.003	1.000	1.000	1.000	1.000	1.000	0.999	1.000	1.000	0.996	0.930	1.000	1.000
Triglyceride	0.804	0.957	0.967	1.000	1.000	1.000	1.000	1.000	1.000	0.986	1.000	0.999	1.000	1.000	1.000
Calcium	1.000	0.998	0.970	0.994	1.000	0.828	0.147	0.554	0.049	0.182	0.999	0.981	0.974	0.093	0.942

Table 4: Comparison between the mean levels of different parameters in group 2 (high-fat diet) compared to different groups according to ANOVA test

Cholesterol	Triglyceride	LDL	HDL	VLDL	Calcium	Phosphorous
0.001	0.063	0.044	0.122	0.014	0.069	0.416

Table 5: Presence or absence of significant differences between the mean levels of Cholesterol, triglyceride and calcium in group2 (high-fat diet) compared to different groups

Parameter	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Cholesterol	0.449	0.000	0.036	0.013	0.015	0.005	0.013	0.079	0.003	0.000	0.119	0.316	0.028	0.003
LDL	0.289	0.433	0.005	0.565	0.219	0.598	0.161	0.130	0.092	0.198	0.861	0.882	0.465	0.974
VLDL	1.000	0.033	0.613	0.859	0.213	0.988	0.406	0.213	0.050	0.439	1.000	0.578	0.374	0.578

Table 6: Comparison between the mean levels of different parameters in groups 3-5 compared to other groups according to ANOVA test

Compared Group	Cholesterol	Triglyceride	HDL	LDL	VLDL	Calcium	phosphorus
3	0.262	0.466	0.246	0.466	0.098	0.137	0.371
4	0.438	0.551	0.223	0.423	0.342	0.166	0.302
5	0.558	0.421	0.174	0.376	0.539	0.177	0.397

DISCUSSION

The results of this study showed that there was a statistically significant difference in mean cholesterol level between the high-fat diet-fed group and the other groups (Tables 4 and 5), but the high-fat diet used did not alter the mean levels of triglycerides, HDL-C, calcium, and phosphorus.

The absence of a statistically significant difference between control group 1 (receiving a normal diet) and other control groups (except control group 2) and treatment groups showed that the consumption of *G. tournefortii* hydroalcoholic extract and kefir along with probiotic yogurts could significantly reduce the amount of increased cholesterol due to the high-fat diet, so that it fell within the range in the group given a normal diet.

Due to the increase in the mean calcium content in group 10, there was a statistically significant difference in mean calcium level between this group (receiving *G. tournefortii* hydroalcoholic extract 0.9%, kefir and 0.6 g *L. acidophilus*) and control group 1 (Table 3). It seems that consuming these compounds together can increase the absorption of calcium in the diet. But the other *G. tournefortii* hydroalcoholic extracts and probiotic

yogurt did not affect serum calcium levels.

Tables 3 and 5 show that there is a statistically significant difference in mean cholesterol level between control group 2 (given high-fat diet) and groups 15, 12, 11, 9, 8, 7, 6, 5, 4, 1 and 16. This indicates that consumption of *G. tournefortii* extract and kefir at the doses administered puts total cholesterol levels in a range that is significantly different from those in groups given high-fat diet.

The lack of a statistically significant difference in mean cholesterol levels between group 2 and group 3 (receiving a high-fat diet and probiotic-free yogurt) indicates that consumption of probiotic-free yogurt cannot significantly prevent the increase in cholesterol induced by a high-fat diet. The statistically significant difference in mean cholesterol level between group 2 and other groups except group 10, indicates the reducing effect of *G. tournefortii* extract, kefir, *L. acidophilus* or *B. bifidum* extract on cholesterol ($P<0.05$).

Besides this, there was no statistically significant difference in mean cholesterol level between group 2 and groups 10 and 13, which in addition to a high-fat diet, kefir and *G. tournefortii* extract 0.6%, were given 0.6 g lyophilized *L. acidophilus* and 0.6 g lyophilized *B. bifidum*, respectively, indicating that consuming kefir with any of the two bacteria lowers cholesterol. However, this reduction did not suffice to yield a significant difference in cholesterol level between these groups and the group receiving the high-fat diet ($P<0.05$). But consumption of kefir with both *L. acidophilus* and *B. bifidum* (0.6 g) and *G. tournefortii* extract 0.6% caused a significant reduction in cholesterol in the group receiving the high-fat diet and a significant statistically difference in mean cholesterol level between groups 2 and 16 ($P<0.05$).

The mean LDL-C level in all groups showed a numerical decrease in comparison to group 2, but this decrease caused a statistically significant difference in this variable only between group 5, which received a high-fat diet with kefir and *G. tournefortii* extract 0.3%, and the group was given the high-fat diet.

It can be therefore argued that in the case of being on a high-fat diet, the ideal diet that can lower LDL-C levels

includes kefir and *G. tournefortii* extract 0.3% (*P*=0.005).

The absence of a statistically significant difference in the mean values of different parameters between groups 3-5 and other groups indicates that consumption of any yogurt, with or without extract, can improve serum cholesterol.

However, the effect of *G. tournefortii* extract and probiotic yogurts together or alone was greater than that of probiotic-free yogurt. Because groups 5-7 received kefir along with *G. tournefortii* hydroalcoholic extract 0.1%, 0.3% and 0.6%, respectively, after receiving a high-fat diet for seven days and groups 8-16 were also given probiotic yogurt, based on our results it can be argued that the effect of *G. tournefortii* hydroalcoholic extract in improving the lipid profile is such that the extract can be used instead of *L. acidophilus* and *B. bifidum*, as there was no statistically significant difference between the groups given kefir and the extract, and the groups receiving kefir, the extract, and the probiotics.

The absence of a statistically significant difference in the mean levels of triglycerides and lipoproteins between group 1 (given normal diet) and other groups indicates that the different dietary patterns used in this study did not suffice to affect these parameters.

Due to the lack of statistically significant differences between the groups that received different doses of *G. tournefortii* extract, the improvement of serum lipid profile is not dose-dependent. Mean triglyceride levels decreased numerically in the groups receiving high-fat diets, probiotics and extracts, but the reduction was not significant statistically.

Given the sharp numerical decrease in triglycerides in some groups compared to the group receiving a high-fat diet, it seems that in the long term a statistically significant difference can yield in mean triglyceride level among the groups receiving high-fat diets, probiotics and extract by increasing the amounts of probiotics and extract.

Regarding the effect of plants on lipid level, Khoshvaghti et al. (2012) reported that consumption of *Zataria Multiflora* hydroalcoholic extracts caused the breakdown of adipose tissue and their transfer to the blood and an increase in fat excretion (16).

Oryan et al. (2010) reported that probiotic yogurt had lower cholesterol and triglyceride levels than probiotic-free yogurt and could lower cholesterol, triglycerides, and LDL-C to a greater extent than HDL-C in the serum of rats consuming the probiotic yogurt (1).

Smith and Adanlawo (2013) reported that dose-dependent consumption of saponin extract reduced increased lipid (17). Mann and Sporry (1974) tested the hypothesis of a cholesterol-lowering effect due to the hydrolyzing activity of probiotics and found that the consumption of probiotic products in the studied samples reduced serum cholesterol, but discontinuation of using these compounds for two weeks caused cholesterol levels to increase (6). Marshal and Tamime (1997) reported that probiotic bacteria eliminated cholesterol with or without the contribution of bile salts (18).There were no statistically significant differences between the serum levels of phosphorous in different groups. This finding showed the consumption of different doses of yogurts containing *G. tournefortii* hydroalcoholic extracts and probiotic yogurts do not have any effects on serum phosphorous.

CONCLUSION

Based on the findings of this study, it can be argued that yogurts containing *G. tournefortii* hydroalcoholic extract have a similar effect to those of probiotic yogurts in hypercholesterolemia induced by the consumption of high-fat diets and the effect does not depend on the dose of the extract.

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