The effect of consuming milk containing Artemia urmiana and Lactobacillus acidophilus on serum lipid profiles in rats fed with high-fat diet

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Abstract

Introduction:
Hyperlipidemia is one of the crucial factors in the occurrence of cardiovascular diseases. The present study aimed to examine the effect of Artemia urmiana on Lactobacillus acidophilus growth in probiotic milk and their effect on serum lipid profiles in rats fed with high fat diet.

Materials and Methods:
To determine effects of different doses (0%, 1%, 2%, 3%) of Artemia urmiana on growth of probiotic bacteria Lactobacillus acidophilus, 0.33 g of lyophilized Lactobacillus acidophilus was separately added to one liter of sterilized low fat milk. Sixty male Wistar rats were then divided into 6 groups of 10, and were fed with different doses of probiotic milk containing Artemia urmiana for 60 days. Blood samples were taken from all the groups at the end of day 60 to measure cholesterol, triglyceride, LDL-C and HDL-C.

Results:
The study results indicated that the increasing concentration of Artemia urmiana has a positive effect on the growth Lactobacillus acidophilus in milk probiotic. Also groups receiving 3 percent of Artemia urmiana powder had the lowest serum cholesterol and the highest serum triglyceride levels.

Conclusion:
Overall Artemia urmiana was found effective on the growth of probiotic Lactobacillus acidophilus in milk. Daily consumption of different doses of Artemia urmiana and lactobacillus acidophilus in milk can improve serum lipid profile through reduction of cholesterol, triglycerides, LDL-C and increasing HDL-C levels.

Keywords: Artemia urmiana, Lactobacillus acidophilus, Lipid profile, Probiotic

Introduction
Despite significant progress in recent years in reducing mortality rate of cardiovascular diseases, these diseases are still considered the primary cause of mortality in many countries (1, 2, 3). Hyperlipidemia is a major risk factor for cardiovascular diseases and among the most common problems of society that both directly and indirectly contributes to the formation of sclerosis plaques and coronary artery diseases by stimulating oxygen free radicals (2). According to studies, increased triglycerides, total cholesterol, and LDL-C levels, and

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decreased HDL-C levels in the blood are among important factors that cause cardiovascular diseases (3). Taking lipid lowering drugs has been the only treatment for these diseases in the past years, but their consumption is dangerous for pregnant women and people with liver and kidney failure. It also leads to complications, such as abdominal pain, allergic reactions, hair loss, headache, muscle atrophy, and reduced libido in healthy people. For example, anethum induces allergic reactions, genotoxicity, and mutations, and gemfibrozil causes gastrointestinal complications, such as diarrhea, constipation, and bloating, and formation of gallstones and myopathy in the long term (2, 3, 4, 5). Modification of diet seems an appropriate and low-risk way to reduce serum lipids. Several studies conducted on laboratory animals and humans have shown that diets containing particular strains of probiotic bacteria can be effective in reducing serum lipids (6). Processed probiotic dairy products are of great importance in promoting health (7). For example, consumption of yogurt, as one of the most important fermented dairy products, has increased over the years due to its health benefits (8). The health benefits of probiotics include antimutagenic and anti-cancer properties, enhancing and modulating the immune system, antimicrobial properties, serum cholesterol reduction, improving lactose intolerance, and enhancing nutritional value (9). Artemia is a valuable nutrient widely distributed on the five continents and Lake Urmia is the largest habitat of Artemia urmiana in the world (10). Artemia has compounds such as fatty acids, protein, and yellow fat (11). The present study aimed to examine the effect of Artemia urmiana on Lactobacillus acidophilus growth in probiotic milk and its effect on serum lipid profiles in rats fed with high fat diet.

Materials and Methods

In order to produce milk containing Lactobacillus acidophilus, 0.33 g of Lactobacillus acidophilus was added to four one-liter containers of sterilized low fat milk (1% fat) as the study groups. Then, 0 (Sham group) 1, 2, and 3% Artemia urmiana powder was added to containers. All containers were incubated at 38 °C. The acidity test was performed approximately every two hours until the acidity reached 42° Dornic. Then, samples were removed from the oven and transferred to a refrigerator at 2 °C. The produced probiotic milk was colony counted every five days by direct counting method (9). After 10 days, milk was evaluated in terms of organoleptic properties. The organoleptic evaluation was performed through questionnaires distributed in a population of 50 people who were asked questions about fragrance, odor, taste, flavor, and consistency at four levels of very good, good, fair, and poor. The data obtained from the questionnaire were analyzed using SPSS-17 and descriptive statistics. In this experimental study, 60 rats with similar physiological structure and environmental conditions were purchased from Animal House of Kazeroon Islamic Azad University. Adult rats had the same age (45 days of age), gender (male), and weight (200-220g), and were housed in the same environmental and nutritional conditions in terms of temperature, humidity, lighting, bedding, feed, and water. Rats were then randomly divided into 6 groups of 10, including controls (no high-fat diet), sham group 1 (receiving high-fat diet and milk), sham group 2 (receiving high-fat diet and milk containing Lactobacillus acidophilus), experimental groups 1, 2, 3 (receiving high-fat diet along with milk containing Lactobacillus acidophilus, 1,2, and 3% Artemia urmiana, respectively). High-fat diet was prepared from feed pellets mixed with animal fats at a ratio of 3:1, and was given to all groups except group 1 (control group) for 60 days. The amount of milk containing Lactobacillus acidophilus and
Artemia urmiana was 30 ml daily for each rat. At the end of the experiment, blood samples were taken from all rats. To this end, the rats were first anesthetized inside a chamber containing ether and blood samples were taken directly from the heart by incising the sternum on both sides. Blood samples were centrifuged at 3500 rpm for 10 minutes to separate the serum for analysis using total cholesterol and triglyceride measurement kits (Zist-Shimi Co.), HDL-C kits (Pars Azmoon Co.), and Biovave Spectrophotometer, (S1 2000, England). The LDL-C level was calculated using Friedewald equation (12). Data were analyzed using SPSS-17 and ANOVA at significance level of p<0.05 (13). Normal distribution of data was examined using the Kolmogorov-Smirnov test.

Results

The acidity level (in Dornic degree) was the highest in the milk containing Lactobacillus acidophilus and 3% Artemia urmiana on the 15th and 20th days of incubation (p<0.05) (Table 1). The milk containing Lactobacillus acidophilus and 1% Artemia urmiana had also the best taste and color in the organoleptic evaluation. Table 2 shows the effect of probiotic milk (sham) and milk containing Lactobacillus acidophilus and Artemia urmiana on cholesterol, triglycerides, LDL-C, and HDL-C levels in rats. In all experimental groups, a significant decrease was observed in cholesterol, triglycerides, and LDL-C levels and an increase in HDL-C levels in rats (p<0.05). In evaluating the microbial culture, Lactobacillus acidophilus grew well on agar. The results indicated that the increasing concentration of Artemia urmiana had a positive effect on the growth of Lactobacillus acidophilus in probiotic milk (Table 3).

Table 1: Changes in the acidity of milk (in Dornic degree) in the incubation period using Lactobacillus acidophilus culture

<table>
<thead>
<tr>
<th>Groups</th>
<th>TC (mg/dl) Mean±SD</th>
<th>TG (mg/dl) Mean±SD</th>
<th>LDL-C (mg/dl) Mean±SD</th>
<th>HDL-C (mg/dl) Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>129.3±4.3</td>
<td>49.85±3.2</td>
<td>78.85±5.6</td>
<td>73.39±3.2</td>
</tr>
<tr>
<td>Receiving milk</td>
<td>180.69±3.9</td>
<td>52.89±3.4</td>
<td>128.25±4.3</td>
<td>40.12±2.20</td>
</tr>
<tr>
<td>with milk</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk containing</td>
<td>183.3±3.2*</td>
<td>43.3±3.4*</td>
<td>76.5±3.2*</td>
<td>42.5±4.4*</td>
</tr>
<tr>
<td>Lactobacillus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>acidoophilus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk containing</td>
<td>140.2±2.3*</td>
<td>44.2±4.2*</td>
<td>77.48±2.3*</td>
<td>43.21±3.1*</td>
</tr>
<tr>
<td>1% Artemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk containing</td>
<td>132.3±2.8</td>
<td>41.32±3.2*</td>
<td>76.8±3.5*</td>
<td>45.2±3.5*</td>
</tr>
<tr>
<td>2% Artemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Indicates a significant increase in acidity in marked groups compared to sham group at P<0.05
Milk containing 3% Artemia & LDL-C, and an increase in HDL-C in marked groups compared to control group 2 at P<0.05.

Table3: Evaluation of microbial growth in milk containing Lactobacillus acidophilus and Artemia urmiana

<table>
<thead>
<tr>
<th>Milk and Artemia</th>
<th>Day 20</th>
<th>Day 15</th>
<th>Day 10</th>
<th>Day 5</th>
<th>Day 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>34×10^7</td>
<td>31×10^6</td>
<td>14×10^6</td>
<td>9×10^6</td>
<td>34×10^6</td>
</tr>
<tr>
<td>1%</td>
<td>39×10^5</td>
<td>26×10^5</td>
<td>42×10^5</td>
<td>18×10^5</td>
<td>21×10^5</td>
</tr>
<tr>
<td>2%</td>
<td>22×10^5</td>
<td>29×10^5</td>
<td>22×10^5</td>
<td>19×10^5</td>
<td>24×10^5</td>
</tr>
<tr>
<td>3%</td>
<td>46×10^10</td>
<td>29×10^10</td>
<td>29×10^10</td>
<td>22×10^10</td>
<td>41×10^10</td>
</tr>
</tbody>
</table>

Discussion
The results showed that the acidity level (in Dornic degree) in milk containing Lactobacillus acidophilus increased with increasing concentration of Artemia urmiana during incubation. Viable probiotic bacteria should be consumed at least 10^10 CFU.ml^-1 to exert their effects (14). Total colony count was higher at a concentration of 3% than that in other concentrations on the 1st and 20th days. Bacteria counts were 10^10 CFU.ml^-1 and colony count was 10^7 CFU.ml^-1 on agar. Given the proven inhibitory and lethal effects of low pH on Lactobacillus acidophilus, the increased shelf life and fermentation process that reduce the pH can be one of the main causes of reducing the growth of Lactobacillus (15). In organoleptic evaluation of milk containing probiotic bacteria and Artemia urmiana, color and taste of the milk were significantly different in different groups (P<0.05) and the milk containing Lactobacillus acidophilus and 1% Artemia urmiana had the best taste and color.

Although the basic property of probiotic products is their medicinal effects (viability), organoleptic properties of these products are also important. In other words, the advantage of taking probiotics as foods not as drugs is their organoleptic properties (15). Producing large amounts of radicals in tissue, high-fat diet increases serum radicals, total cholesterol, triglycerides, and LDL-C levels, and decreases HDL-C levels which might lead to coronary heart diseases, as one of their consequences (16). The results also showed that daily and long-term consumption of milk containing Lactobacillus acidophilus and Artemia urmiana significantly reduces the mean serum total cholesterol, triglycerides, and LDL-C levels (P<0.05) and increases the mean HDL-C (P<0.05). Zhao (2005) reported that of the total 21 Lactobacillus and Bifidobacterium strains isolated from adolescents and young adults’ stool specimens, six strains were able to remove cholesterol from the culture medium in vitro (17). According to Ashar’s study (2000) conducted on 27 hyperlipidemic patients, daily consumption of 200 ml of milk containing Lactobacillus acidophilus for 20 days significantly reduced serum total cholesterol (18). According to Park et al. study (2007) on 36 rats, a diet containing Lactobacillus acidophilus ATCC43121 reduced serum total cholesterol by 25%, but did not change the liver tissue cholesterol (19). Chiu et al. (2006) also found that milk fermented by three strains of Lactobacillus reduced serum and hepatic lipid levels in hamsters fed with high fat diet by about 30.1% and 13.4%, respectively (20).

Conclusion
In general, according to the results of this study and other studies, it can be stated that the consumption of milk containing Lactobacillus acidophilus and Artemia urmiana improves serum lipid profile and can be a good diet by reducing total cholesterol, triglycerides, and LDL-C levels, and increasing HDL-C levels. Undoubtedly, generalizing the results of this study to humans requires extensive and specialized studies on this nutrient.
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Conflict of Interest
The authors declare no conflicts of interest regarding the compilation/ publication
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