The Effect of Probiotic (Lactobacillus acidophilus) on the Absorption of Calcium and Cholesterol in The intestines of Rats

Mohammed karee jabbar*

Faculty of pharmacy, Humanity of studies university college, Iraq.

Abstract: The interaction of Lactobacillus acidophilus with some physiological aspects of intestinal in regard to intestinal mucosal cells proliferation, differentiation and absorption were studied. The present study designed to study the influence of LBA on intestinal absorption. Therefore, male Swiss albino rats and divided into two main groups of rats each group consist of five rats, - first group: this Control (C) group received (1 ml per animal) of distilled water by oral gavages tube for 4 week. Second group: this treated (T) group received (5*10 CFU) Lactobacillus acidophilus as probiotics by oral gavages tube for 4 week and each group was fed on a balanced diet with high calcium (1.8%) from calcium citrate. The experiment continued for 30 days. At the end of experiments, all experimental animals were sacrificed. Serum levels of calcium and cholesterol measurement.

Results of the present experiment revealed that there was significant difference in levels of calcium in C, T and the levels of calcium which was significantly (p<0.05) increased in rats of T compared with C, the results showed significant decrease in lipid profile (p<0.05) in T group compared with C group.

Introduction

Cardiovascular disease due to atherosclerosis of the arterial vessel wall and to thrombosis is the foremost cause of premature mortality and of disability-adjusted life years in Europe and is also increasingly common in the developing countries [1]. Hyperlipidaemia is a dominant risk factor for cardiovascular diseases and the leading cause of death in many countries. Elevated serum cholesterol is generally risk factor correlated with the development of coronary artery diseases. Dietary fat is one of the most important environmental factors associated with the incidence of these diseases; diets high in cholesterol and saturated fat have been shown to promote atherosclerosis [2]. Atherosclerosis considered to be a modified form of chronic inflammation induced by lipids and many have followed in this path including evidence that numerous cellular adhesion molecules and growth factors were determined in the atherosclerotic plaques [3]. The current drug therapy has the disadvantage owing to its undesirable side effects and cost, so there is an increasing interest in alternative approaches to lower cholesterol [4]. Diet intervention supplements have now been extensively studied to reduce risk factor for cardiovascular disease. Numerous animal experiments and human studies have reported that probiotic microorganisms display hypolipidemic effects by inhibiting cholesterol biosynthesis and decreasing low density lipoproteins [5, 6]. Food and Agriculture Organization and World Health Organization defined probiotics as “Living microorganisms which when administered in adequate amounts confer a health benefit on the host” [7]. There is currently adopted definition by FAO/WHO, but in the present research publications there are also new definitions of probiotics as follows: probiotics are living microorganisms which
modulate the specific function of organism by activation of specific molecular pathways [8]. The gastrointestinal tract is one of the largest interfaces between the outside world and the human or animal internal environment.

II. Material and Methods

Ten health Albino Wister pregnant male rats, were kept under suitable condition of (21-25°C) in an air condition room, they were fed freely with standard pellet diet. The animals were divided into two main groups. **first group**: this Control (C) group received (1 ml per animal) of distilled water by oral gavages tube for 4 weeks. **Second group**: this treated (T) group received (5×10^9 CFU) Lactobacillus acidophilus as probiotics by oral gavages tube for 4 week. And each group was fed on a balanced diet with high-calcium (1.8%) from calcium citrate.

<table>
<thead>
<tr>
<th>Table 1. Diet composition (%) of experimental rats</th>
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<tbody>
<tr>
<td>Component</td>
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<tr>
<td>-----------</td>
</tr>
<tr>
<td>Proteins</td>
</tr>
<tr>
<td>Fats</td>
</tr>
<tr>
<td>Carbohydrates</td>
</tr>
<tr>
<td>Celloside</td>
</tr>
<tr>
<td>Minerals</td>
</tr>
<tr>
<td>Vitamins</td>
</tr>
<tr>
<td>Calciumcitrate</td>
</tr>
<tr>
<td>Dryskinned milk</td>
</tr>
<tr>
<td>Hydroxyapatite</td>
</tr>
</tbody>
</table>

Diet composition (%) of different rat subgroups is shown in Tables (1-2).

At the end of the experiment all the experimental animals were anesthetized by intraperitoneal injection with 35 mg/kg ketamine hydrochloride. After opening the chest cavity, blood was collected by acupuncture through the left ventricle. Blood samples were collected twice; at the beginning and at the end of experimental period (after 4 weeks). The first blood samples were collected from Jugular vein of rats. Second blood samples were collected from animals’ hearts after anesthesia and sacrificing at the end of the 4th week.

**Laboratory Analysis**

1-Determination of lipid profile in serum

The blood serum was used for determination of total cholesterol (TC), high density lipoproteins cholesterol (HDL-C), and triglycerides (TG) were measured by using an automatic biochemical analytical system. Low density lipoprotein (LDL-C) was calculated by Friedewald formula (W. T. Friedewald et al., 1982)

2-Determination of calcium in serum

Serum calcium was determined according to the method of Gindler and King (1972).

**a. Principle**

1. Calcium ion produces with methylthymol blue, in an alkaline medium, a blue color the intensity of which is in proportion to 40
2. the calcium concentration. The presence of hydroxyl 8-quinoline eliminate the interference due to magnesium ions.

**b. Reagents**

1. Standard: (10 mg/dl = 2.5 mmol/l).
2. Chromogen: methylthymol blue (0.2 mmol/l), hydroxyl 8-quinoline (140 mmol/l) and hydrochloric acid (200 mmol/l).

c. Procedure
1. Equal volumes of reagent 1 and reagent 2 were mixed well before use.
2. An aliquot from each sample (20 μl) or standard was transferred to clean tubes.
3. Portions of working reagent (1 ml) were added to sample, blank and standard tubes.
4. All tubes were well mixed and incubated for 5 min at room temperature.
5. The absorbance of sample and standard were measured against the blank at 585 nm.

d. Calculation

Serum calcium concentration (mg/dl) = A sample / A standard × 10

Statistical Analysis

The Statistical Analysis System- SAS (2010) was used to effect of different factors in study parameters. Least significant difference –LSD test was used to significant compare between means in this study.

III. Results

Results of serum calcium and lipid profile level in initial experiment in table (1) show that was not significantly in T group compare with control group while results of serum calcium and lipid profile level in final experiment in table (2) show that was significantly increase (p<0.05) in T group compare with control group while results of serum lipid profile level was decrease significantly (p<0.05) in T group compare with control group.

Table 1. Lipid profile and calcium in initial experiment of rats.

<table>
<thead>
<tr>
<th>Rat group</th>
<th>Serum calcium (mg/dl)</th>
<th>Cholesterol (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>8.9±0.3a</td>
<td>79.3±2.1</td>
<td>66.9±1.5</td>
<td>40.2±0.5</td>
<td>121.0±3.5</td>
</tr>
<tr>
<td>T group</td>
<td>9.2±0.6b</td>
<td>78.5±3.2</td>
<td>67.5±1.2</td>
<td>41.5±0.3</td>
<td>120.9±2.5</td>
</tr>
</tbody>
</table>

Table 2. Lipid profile and calcium in final experiment of rats.

<table>
<thead>
<tr>
<th>Rat group</th>
<th>Serum calcium (mg/dl)</th>
<th>Cholesterol (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>9.3±0.3a</td>
<td>80.3b±2.1</td>
<td>65.9b±1.5</td>
<td>39.5b±0.5</td>
<td>138.4b±3.5</td>
</tr>
<tr>
<td>T group</td>
<td>13.2±0.6b</td>
<td>74.5±3.2</td>
<td>61.5a±1.2</td>
<td>37.5a±0.3</td>
<td>128.9a±2.5</td>
</tr>
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IV. Discussion

This study was based on the hypothesis that the addition of lactobacillus will alter the intestinal development along with growth leading to changes intestinal morphology and effect on absorbed of the intestinal.

It has been reported that lactobacillus increase the calcium absorption from intestinal tract. There are more than mechanical explain the increased absorption of calcium from the intestine (Gilman et al., 2006). Fermentation products produced by Lactobacillus increase the surface area of the intestine by accelerating proliferation in enterocytes (Scholz-Ahrens at al., 2007). Awad et al. (2010) reported that supplementation of
Lactobacillus acidophilus on intestinal morphology and cell proliferation lead to increased villus height and villus height and crypt depth ratio. Furthermore short chained fatty acids and the other products produced by the bacteria decrease the pH of intestine microenvironment. Therefore, calcium solubility increases and this may be related to increased calcium absorption (Gilman et al., 2006; Scholz-Ahrens at al., 2007).

This study has shown the effect of Lactobacillus acidophilus on intestinal absorption of cholesterol, Where it reduces the absorption of cholesterol in the intestine due to the consumption of Lactobacillus acidophilus cholesterol for growth, the binding of cholesterol into surface of Lactobacillus, thereby inhibiting the absorption of cholesterol back into the body. The deconjugation of bile acids by bacterial acid hydrolases, increasing cholesterol excretion of deconjugated bile salts and increasing cholesterol uptake and metabolism in the liver as compensatory response because bile acids are synthesized from cholesterol in the liver and inhibition of hepatic cholesterol and triglyceride synthesis through the action of short chain fatty acids, especially propionic acid (M. T. Liong and N. P. Shah, 2006; D. O. Noh, S. H. Kim, and S. E. Gilliland, 1997).

References