Effect of fermented camel milk and cow milk containing (bifidobacteria) enriched diet in rats fed on cholesterol level

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Abstract

The effect of fermented camel milk Gariss and fermented cow milk +Bifidobacteria on plasma on liver lipids was determined in rats fed with cholesterol enriched diet. The two groups were decreased the levels of plasma total cholesterol, Very Low-Density Lipoprotein (VLDL) and Low-Density Lipoprotein (LDL) cholesterol than the positive control group. Gariss was more effective in the lowering of plasma and liver cholesterol levels than fermented cow milk +Bifidobacteria.

Key words: Cholesterol, fermentation, camel milk, plasma lipids.

INTRODUCTION

For a long time, milk was considered to only provide nutritional components such as essential amino acids (Hambræus, 1992). In the last decades, several studies have shown that milk is an important nutritional and functional source and could provide particular health benefits due to the presence of bioactive substances in milk. Fresh and fermented Dromedary camel milk have been acknowledged for a long time in different parts of the world to provide a potential treatment for a series of diseases such as dropsy, jaundice, tuberculosis, asthma, and leishmaniasis or kala-azar (Abdelgadir et al., 1998; Shalash, 1984). These potential health benefits are obtained through a number of bioactive components in camel milk. These components were reported to exist naturally in camel milk (Agrawal et al., 2007a; El-Agamy, et al. 1992); or derived from camel milk proteins using probiotic strains (Elayan et al., 2008; Quan et al., 2008).

Coronary heart disease is one of the major causes of death in the industrialized countries (Pereira & Gibson, 2002). Elevated levels of blood and dietary cholesterol are considered to be a major risk factor for coronary heart diseases (Reddy, et al, 1977).

Fermented camel milk (Gariss) and Gariss containing Bifidobacterium lactis (BB-12) administration have been reported to possess a hypocholesterolaemic effect in vivo in rats (Elayan et al 2008) This strain was previously shown to reduce cholesterol in bovine milk and MRS broth (Alhaj et al., 2006). The hypocholesterolaemic mechanism of camel milk is still unclear, but different hypotheses have been proposed including: interaction between bioactive peptides derived from camel milk proteins and cholesterol which result in cholesterol reduction (Li & Papadopoulos, 1998; Seelig & Seelig, 1996). Fermented dairy products have been recommended as dietary supplements because of their hypocholesterolaemic effect in humans (Mann, 1977) and rats (Suzuki et al., 1991). It was reported that buffalo milk yoghurt or soynilk yoghurt containing bifidobacterium reduced the level of plasma and liver cholesterol (Abdel Gawad et al., 1998).

Therefore, the objective of this study was to detect the hypocholesterolaemic effect of Gariss and fermented cow milk containing bifidobacterium on the levels of plasma and liver lipids of rats.
Table 1. The experimental rats groups and their diets

<table>
<thead>
<tr>
<th>Diet treatment</th>
<th>Diet code</th>
<th>Diet formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol free diet</td>
<td>Negative</td>
<td>100 g basal diet+50ml water</td>
</tr>
<tr>
<td>Cholesterol – Enrich Diet(CED)</td>
<td>Control</td>
<td>99.5g basal diet+0.5 g cholesterol+50ml water</td>
</tr>
<tr>
<td>CED + Gariss fermented camel milk</td>
<td>Positive control</td>
<td>99.5 g basal diet+0.5 g +0.5 g cholesterol+50g Gariss</td>
</tr>
<tr>
<td>CED + Fermented cow milk</td>
<td>C+Bnb-12</td>
<td>Fermented cow milk</td>
</tr>
</tbody>
</table>

basl diet =cholesterol-free diet
CED =cholesterol-enriched diet

Materials and Methods

Animal Feeding Experiment

Twenty four albino rats with an average weight between 80 and 100g were house at 23±1oC and 60±5% relative humidity. All animals were fed on basal diet (cholesterol-free diet) for one week. After this adoption period, the rats were divided randomly into 4 experimental groups; one group received a basal diet throughout the experimental period for 6 weeks and served as a negative control group. The other three groups were fed on basal diet with cholesterol added at a level of 0.5% (w/w) cholesterol-enriched diet) for 1-2 weeks to create hypercholesterolaemic rats. One of the three groups, which served as positive control group, was fed only on a cholesterol enriched diet for the six-week period.

The other 2 groups were fed for five weeks on a cholesterol-enriched diet supplemented with G and fermented cow milk. The experimental diet given to the 4 groups are described in Table 1. The rats were allowed free access to experimental diet and water and their body weights were monitored, at the end of the 6 weeks experimental period. Blood samples were collected from the eye vein under diethyl ether anesthesia. The samples were collected in tubes, with EDTA as an anti-coagulating agent. The tubes were centrifuged at 3000 rpm for 15 min to obtain the plasma, which was kept frozen (-23±1oC) until analysis.

The tubes were centrifuged at 3000 rpm for 15 min to obtain the plasma, which was kept frozen (-23±1oC) until analysis.

The rats were sacrificed and the liver, heart, kidney and spleen were excised immediately and weighed. The liver was washed with ice-cold saline solution (0.9 w/v,NaCl) and stored at 23±±1oC until analyzed.

The basal diet consisted of 5% (w/w), casein 10% (w/w), maize oil 10% (w/w), cellulose 4% (w/w), mineral mix 1% (w/w), vitamin mix 1% and starch 60% (w/w).

Determination of Plasma Lipid

Total plasma cholesterol, High Density Lipoprotein (HDL) and triglycerides determined using the enzymatic colorimetric method according to Trinder (1969). The VLD+LDL cholesterol= Total cholesterol – HDL cholesterol.

Determination of Cholesterol and Triglycerides in Liver

Cholesterol and triglycerides were extracted from the liver by the method of Fernandez et al. (1997) and were measure by the method of Trinder (1969).

Statistical Analysis

Dates are presented as means and standard deviations. The significance between groups/treatments was evaluated using general linear model procedure of Statistical Analysis Systems (SAS) (1990). The biological examination data was analyzed by least Significant Difference (LSD) at p < 0.05 (SAS, 1990).

RESULTS AND DISCUSSION

Plasma and Lipids

The effect of on liver triglyceride, total cholesterol and 7 keto-cholesterol in rats fed on cholesterol enriched diets were presented in Table (2). The total concentration of plasma cholesterol was significantly reduced from 135.7 mg/100Ml in the positive control to the mean value of 87.9mg/100Ml in both Garris and Cow-Bb-12groups. This decrease corresponds to 35.3 and 35.2% reduction, respectively. In contrast, there was no significant difference in total cholesterol detected in (Garris and Cow milk-Bb-12) and negative control groups. Anderson and Gilland (1999) found that every 1% reduction in serum cholesterol concentration is associated with an estimated 2% to 3% reduction in risk coronary heart disease, regular intake of fermented milk containing an appropriate strain of L. acidophilus has potential of reducing risk for coronary heart disease by 6 to 10%.

It was noticed cholesterol-enriched diet (supplemented with Cow milk-Bb-12) lowered total cholesterol with the same efficiency of Gariss. Abdel Gawad et al. (1998)
reported that cholesterol –enrich diet supplemented with yoghurt containing bifidobacteria were more effective at lowering total cholesterol.

Beena and Prasad (1997) found lower serum cholesterol in rats fed on yoghurt containing Bifidobacterium bifidum (120 mg/100 ml) compared to a positive control (176 mg/100 ml) after 30 days. However, Homma (1998) found that fermented milk containing bifidobacteria (10 cfu g-1) resulted in a decrease in the total cholesterol level from 300 to 150 mg/100ml) in human subject. Moreover, Schammann et al. (2001) reported that the consumption of the probiotic yoghurt made with B. longum and L. acidophilus decreased total cholesterol in hypercholesterolaemic women from 293 mg/100 Ml at the beginning of experiment to 255 mg/100ml after 153 days. Fermented camel milk (Gariss) and Gariss containing Bifidobacterium lactis (BB-12) administration have been reported to possess a hypocholesterolaemic effect in vivo in rats (Elayan et al., 2008). This strain was previously shown to reduce cholesterol in bovine milk and MRS broth (Alhaj et al., 2006) as well as in trypitcose-peptone yeast extract medium (Tahri, et al, 1995). The hypocholesterolaemic mechanism of camel milk is still unclear, but different hypotheses have been proposed, including: interaction between bioactive peptides derived from camel milk proteins and cholesterol which result in cholesterol reduction (Li and Papadopoulos, 1998; Seelig and Seelig, 1996), and the presence of orotic acid in camel milk which is thought to be responsible for lowering cholesterol level in human subjects (Buonopane, et al, 1992) and in rats (Rao, et al, 1981).

As shown in Table 3, there was no significant difference in the plasma HDL-cholesterol level between the negative control group and other experimental groups at the end of the 6week experimental period. Endo et al. (1999) reported that the addition of the probiotic to diet had no effect on the HDL-cholesterol level. Our data agree well with this finding.

Rats fed on (Garris) and (Cow milk+Bb-12) diet significantly lowered plasma VLDL+LDL-cholesterol than positive control group of rats. The G diets more effective in lowering plasma VLDL+LDL-cholesterol levels than (Cow milk+Bb-12). Beena and Prasad (1997) found that yoghurt containing B. bifidum markedly lowered the levels of LDL-cholesterol in rats fed on a cholesterol-enriched diet (positive control) from 97mg/100 mL to 22.16 and 15mg/100ml respectively, in rats fed on yoghurt containing B. bifidum fortified with skimmed milk, condensed whey or lactose-hydrolysed condensed whey at the end of the 30 days experimental period. Moreover, Schaammann et al. (2001) reported that the consumption of probiotic yoghurt made using B. longum and L. acidophilus reduced the LDL –cholesterol level in normcholesterolaemic and cholesterolamemic women. The levels of plasma triglycerides in rats fed on (Garris) and (Cow milk+Bb-12) diets were significantly. Lower than that in those fed the cholesterol-enriched diet (positive control). The reduction in triglyceride levels with the above mentioned groups was 52.6 and 33.1, respectively (Table 3). Schaarmann et al.(2001) reported that the consumption of probiotic yoghurt made with B. longum and L. acidophilus lowered triglycerides in hypercholesterolaemic women from 114 mg/100 Ml to 91 mg/100 Ml after 153 days.

The data in Table 4 show that the content of liver cholesterol in the positive control group (4.94mg-1) was significantly higher than in the negative control group (2.38mg-1). In addition, there was a significant difference between rats feed on the positive control diet and those fed on (Garris) and (Cow milk+Bb-12) diets. It can be observed that fermented camel milk was effective in lowering to the levels of liver cholesterol. These results are in agreement with those reported by Kheadr et al. (2000) who suggested that the yoghurt diets supplemented with B. bifidum reduced cholesterol in rat liver tissues.

The content of liver triglycerides was significantly higher in rats fed on the positive control diet than those fed the negative control diet (Table 4). In contrast, the levels of liver triglycerides in rats fed on the experimental diet groups were significantly lower than in rats fed on the positive control diet. The value of body weight gain was significantly higher in rats fed (Garris) and (Cow milk+Bb-12) than that in rats fed the negative control diet. The body weight gain in rats fed (Cow milk+Bb-12) diet was nonsignificantly higher than that in rat fed the positive control diet (Table 4). No significant difference in weights of heart or were detected between rats fed on the (Garris) and (Cow milk+Bb-12) diets and rats fed the two control diets. There was a significant difference in kidney weight between rats fed the negative control diet and rats fed other diets. The liver weight in rats fed the positive control diet was significantly higher than rats fed negative control diet. There was a significant difference in the liver weight between rats fed the positive control diet and rats fed the other experimental diets.

Conclusions

This study has concluded that a cholesterol-enriched diet of rats containing fermented camel milk and fermented cow milk with bifidobacteria had a market effect level of plasma and liver lipids. Fermented camel milk and fermented cow milk containing bifidobacterium Bb-12 were very effective in lowering the level of plasma and liver lipids in rats. These hypocholesterolaemic effects of Gariss and fermented cow milk containing bifidobacterium Bb-12, which have been demonstrated in the rats in the present study, could make an effective and economic contribution in treating hypercholesterolaemic if these effects could be confirmed in human volunteers.
Table 2. Effect of on liver triglyceride, total cholesterol and 7 keto-cholesterol in rats fed on cholesterol enriched diet

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Tryglyceride (mg g⁻¹)</th>
<th>Total cholesterol (mg g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control</td>
<td>25.22±2.87</td>
<td>4.94±0.47</td>
</tr>
<tr>
<td>Negative control</td>
<td>19.71±0.46</td>
<td>2.38±0.27</td>
</tr>
<tr>
<td>Fermented camel milk</td>
<td>18.32±3.90</td>
<td>2.73±0.21</td>
</tr>
<tr>
<td>Fermented cow milk</td>
<td>20.16±3.07</td>
<td>2.69±0.38</td>
</tr>
</tbody>
</table>

Table 3. Effect on serum lipid profile in rats fed on cholesterol enriched diet

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Triglyceride (Mg 100Ml⁻¹)</th>
<th>Total cholesterol (Mg 100Ml⁻¹)</th>
<th>HDL (Mg 100Ml⁻¹)</th>
<th>VLDL+LDL (Mg 100Ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control</td>
<td>144.27±4.47</td>
<td>135.79±8.74</td>
<td>11.66±1.29</td>
<td>124.13±7.55</td>
</tr>
<tr>
<td>Negative control</td>
<td>77.28±3.64</td>
<td>109.23±5.77</td>
<td>36.12±1.88</td>
<td>73.10±4.93</td>
</tr>
<tr>
<td>Fermented camel milk</td>
<td>68.25±3.30</td>
<td>87.93±4.00</td>
<td>28.78±1.07</td>
<td>59.16±2.94</td>
</tr>
<tr>
<td>Fermented cow milk</td>
<td>68.25±3.30</td>
<td>87.95±9.48</td>
<td>29.87±1.04</td>
<td>58.07±8.60</td>
</tr>
</tbody>
</table>

Table 4. Effect on bodyweight gain and weight of liver, heart and kidney in rats fed on cholesterol enriched diet

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Body gain (g)</th>
<th>Liver (g)</th>
<th>HEART (g)</th>
<th>Kidney (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control</td>
<td>20.5±15.87</td>
<td>7.68±0.56</td>
<td>0.69±0.05</td>
<td>1.30±0.15</td>
</tr>
<tr>
<td>Negative control</td>
<td>17.10±12.25</td>
<td>3.95±0.28</td>
<td>0.42±0.06</td>
<td>0.89±0.01</td>
</tr>
<tr>
<td>Fermented camel milk</td>
<td>25.27±5.32</td>
<td>6.02±0.89</td>
<td>0.49±0.06</td>
<td>1.18±0.12</td>
</tr>
<tr>
<td>Fermented cow milk</td>
<td>26.25±1.69</td>
<td>6.11±0.26</td>
<td>0.50±0.08</td>
<td>1.09±0.09</td>
</tr>
</tbody>
</table>

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