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The Hypocholesterolemic Effect of *Gariss* and *Gariss* Containing Bifidobacteria in Rats Fed on a Cholesterol-Enriched Diet

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Abstract: The effect of fermented camel milk *Gariss* and *Gariss* supplemented with *Bifidobacterium lactis* (Bb-12) on plasma and liver lipids was determined in rats fed on a cholesterol enriched diet. The two groups decreased the levels of plasma total cholesterol and the Very Low-Density Lipoprotein (VLDL) and Low-Density Lipoprotein (LDL) cholesterol than the positive control group. *Gariss* containing Bb-12 was more effective in the lowering of plasma and liver cholesterol levels than *Gariss* without bifidobacterium.

Key words: Cholesterol, fermented dairy products, Bifidobacteria, plasma lipids, triglycerides, statistical analysis

INTRODUCTION

Hypercholesterolaemic is a major risk factor associated with coronary heart diseases and it is considered that keeping blood cholesterol at a desirable level is one of the major preventive strategies for these diseases. Thus, much attention has been given to the relationship between diet and blood cholesterol levels.

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 soymilk yoghurt containing bifidobacterium reduced the level of plasma and liver cholesterol (Abdel Gawad *et al*., 1998).

Relatively little is yet known regarding the potential role of the health promoting effects of *Gariss* and *Gariss* containing bifidobacteria by reducing cholesterol. Therefore, the objective of this investigation was to detect the hypocholesterolaemic effect of *Gariss* and *Gariss* containing bifidobacterium on the levels of plasma and liver lipids of rats.

MATERIALS AND METHODS

Animal Feeding Experiment

Twenty four albino rats with an average weight between 80 and 100 g were house in cages with wiremesh floors in a room at 23±1°C and 60±5% relative humidity. All animals were fed on basal diet for one week. After this adoption period, the rats were divided randomly into 4 experimental groups of 6, one group received a basal diet (cholesterol-free diet) throughout the experimental period of 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.

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Table 1: Experimental groups of rats and diets

Diet treatment	Diet code	Diet formulae
Cholesterol free diet	Negative control	100 g basal diet+50 mL water
Cholesterol-Enrich Diet (CED)	Positive control	99.5 g basal diet+0.5 g cholesterol+50 mL water
CED+*Gariss* fermented camel milk	G	99.5 g basal diet+0.5 g+0.5 g cholesterol+50 g *Gariss*
CED+*Gariss* with added *Bifidobacterium lactis* Bb-12	G+Bb-12	99.5 g basal diet+0.5 g cholesterol+50 g *Gariss*+Bb-12

The other 2 groups were fed for five weeks on a cholesterol-enriched diet supplemented with G and G+Bb-12. The experimental diets 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±1°C) 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±1°C until analyzed.

The basal diet consisted of 15% (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 were determined using the enzymatic colourimetric method according to Trinder (1969). The VLDL+LDL was calculated as follows:

$$\text{VLDL+LDL cholesterol} = \text{Total cholesterol} - \text{HDL cholesterol}.$$

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 measured by the method of Trinder (1969).

Statistical Analysis

Data are presented as means and standard deviations. The significance differences between groups/treatments were 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 the experimental diets on the levels of plasma and liver lipids in rats are presented in Table 2-4.

The total concentration of plasma cholesterol was significantly reduced from 135.7 mg/100 mL in the positive control to the a mean value of 87.9 mg/100 mL in both G and G-Bb-12 groups. This decrease corresponds to 35.3 and 35.2% reduction, respectively. In contrast, there was no significant difference in total cholesterol detected in (G and G-Bb-12) and negative control groups.

It was noticed cholesterol-enriched diet (supplemented with G-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.

Table 2: Effect of bifidobobacterial *Gariss* on liver triglyceride, total cholesterol and 7 keto-cholesterol in rats fed on cholesterol enriched diet

Treatments	Tryglyceride (mg g^{-1})	Total cholesterol (mg g^{-1})
Positive control	25.22±2.87^{a}	4.94±0.47^{a}
Negative control	19.71±0.46^{b}	2.38±0.27^{b}
Gariss 1	18.32±3.90^{b}	2.73±0.21^{b}
Gariss 2	20.16±3.07^{ab}	2.69±0.38^{b}

Table 3: Effect of biofidobacterial *Gariss* on serum lipid profile in rats fed on cholesterol enriched diet

Treatments	Triglyceride (Mg 100 mL^{-1})	Total cholesterol (Mg 100 mL^{-1})	HDL (Mg 100 mL^{-1})	VLDL+LDL (Mg 100 mL^{-1})
Positive control	144.27±4.47^{a}	135.79±8.74^{a}	11.66±1.29^{c}	124.13±7.55^{a}
Negative control	77.28±3.64^{c}	109.23±5.77^{b}	36.12±1.88^{a}	73.10±4.93^{b}
Gariss 1	68.25±3.30^{c}	87.93±4.00^{c}	28.78±1.07^{a}	59.16±2.94^{c}
Gariss 2	96.47±14.06^{b}	87.95±9.48^{c}	29.87±1.04^{b}	58.07±8.60^{c}

Table 4: Effect of bifidobobacterial *Gariss* on body weight gain and weight f liver, heart and kidney in rats feed on cholesterol enriched diet

Treatments	Body gain	Liver	Heart	Kidney
Positive control	20.54±15.87^{a}	7.68±0.56^{a}	0.69±0.05^{a}	1.30±0.15^{a}
Negative control	17.10±12.25^{a}	3.95±0.28^{c}	0.42±0.06^{b}	0.89±0.01^{c}
Gariss 1	25.27±5.32^{a}	6.02±0.89^{b}	0.49±0.06^{b}	1.18±0.12^{ab}
Gariss 2	26.25±1.69^{a}	6.11±0.26^{b}	0.50±0.08^{b}	1.09±0.09^{b}

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 (172 mg/100 mL) after 30 days. However, Homma (1998) found that fermented milk containing bifidobactria (10^{9} cfu g^{-1}) resulted in a decrease in the total cholesterol level from 300 to 150 mg/100 mL) in human subject. Moreover, Schaarmann *et al*. (2001) reported that the consumption of the probiotic yoghurt made by *B. Longum* and *Lactobacillus acidophilus* decreased total cholesterol in hypercholesterolaemic women from 293 mg/100 mL at the beginning of experiment to 255 mg/100 mL after 153 days.

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 6-week experimental period. Endo *et al*. (1999) reported that the addition of the probiotic to diet had on effect no the HDL-cholesterol level. Our data agree well with this finding.

Rats fed on (G) and (G+Bb-12) diet significantly lowered plasma VLDL+LDL-cholesterol than positive control group of rats. The (G+Bb-12) diets were more effective in lowering plasma VLDL+LDL-cholesterol levels than (G). 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 97 mg/100 mL to 22.16 and 15 mg/100 mL, 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, Schaamann *et al*. (2001) reported that the consumption of probiotic yoghurt made using *B. longum* and *L. acidophilus* reduced the LDL-cholesterol level in normocholesterolaemic and cholesterolamemic women.

The levels of plasma triglycerides in rats fed on (G) and (G+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. acidphilus* 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 (4.94 mg g^{-1}) was significantly higher than in the negative control group (2.38 mg g^{-1}). In addition,

there was a significant difference between rats fed on the positive control diet and those fed on (G) and (G+Bb-12) diets. It can be observed that *Garris* was effective in lowering 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 (G) and (G+Bb-12) than that in rats fed the negative control diet. The body weight gain in rats fed (G+Bb-12) diet was non-significnatly 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 (G) and (G+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 demonstrated that the inclusion of *Gariss* and *Gariss* with bifidobacteria in the diet of rats fed a cholesterol-enriched diet had a marked effect on the level of plasma and liver lipids. *Gariss* and *Gariss* containing bifidobacterium Bb-12 were very effective in lowering the level of plasma and liver lipids in rats. These hypercholesterolaemic effects of Gariss and Gariss containing bifidobacterium Bb-12, which have been demonstrated in the rats in the present study, could make an effective and economic contribution in treating hyperchloesterolaemic if these effects could be confirmed in human volunteers.

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