Effect of Dahi Containing *Lactococcus lactis* on the Progression of Diabetes Induced by a High-Fructose Diet in Rats

Hariom Yadav, Shalini Jain, and P. R. Sinha

Animal Biochemistry Division, National Dairy Research Institute, Karnal-132001, Haryana, India

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The antidiabetic effect of dahi was observed on high-fructose-induced diabetic rats. The fasting blood glucose, glycosylated haemoglobin, insulin, free fatty acids and triglyceride levels of the dahi fed group animals were significantly lower than those of the control group \((p < 0.05)\). The imparity of the glucose tolerance test was also delayed by one week in the dahi-fed animals.

Key words: dahi; *Lactococcus*; diabetes; antidiabetic effect; glucose tolerance

Dahi has anecdotally been recommended for consumption by healthy people to lower the risk of chronic diseases in Asian countries. In these countries, dahi is provided to patients for the clinical treatment of gastrointestinal and metabolic diseases, hypertension, ischemic heart disease and allergy. Although contradictory results have been obtained, the majority of results from these reports indicated that fermented dairy products, possibly including dahi, possessed hypocholesterolemic properties. The consumption of fermented milk has the potential to reduce blood cholesterol levels and lower the serum cholesterol concentration in animals and humans. Although contradictory results have been obtained, the majority of results from these reports indicated that fermented dairy products, possibly including dahi, possessed hypocholesterolemic properties. The consumption of fermented milk has the potential to increase gut bacterial colonization via the protective effect of milk protein for survival through the gastrointestinal transit. However, antidiabetic effects have not been studied for fermented milk products.

The aim of the present study was therefore to determine whether dahi supplementation would alter the glucose tolerance test and the glycosylated haemoglobin, insulin, triglyceride and fatty acid levels in a mildly high-fructose diet-induced diabetes model in rats.

The dahi culture containing *Lactococcus lactis* ssp *lactis* biovar *diacetyl lactis* NCDC 60 was obtained from National Collection of Dairy Cultures (NCDC) at this institute. Raw buffalo milk was procured from the National Collection of Dairy Cultures (NCDC) at this institute. Raw buffalo milk was procured from the National Collection of Dairy Cultures (NCDC) at this institute. The fat level being adjusted to 2.5% by adding fresh skim milk. The milk was boiled at 90 °C for 15 min and then cooled to 37 °C. Dahi was prepared by inoculating the NCDC 60 culture for 12–14 hrs. The rats were randomly divided into two groups: i) the Normal group \((n = 6)\) fed with a standard diet without fructose and dahi; ii) Control group \((n = 6)\) fed with a high-fructose diet (Table 1); and iii) Dahi fed group \((n = 6)\) fed with a dahi-supplemented high-fructose diet for 42 days. Water was supplied *ad libitum*. The protein contents and caloric values of the three diets were similar (Table 1). The daily food intake and body weight were analysed at weekly intervals. This study was approved by the National Dairy Research Institute Animal Ethics Committee, and the rats were maintained in accordance with the National Institute of Nutrition, India guidelines for the care and use of laboratory animals.

The oral glucose tolerance tests (OGTT) were performed at weekly intervals during the experimental period on 12-h-fasted rats before the administration of an oral glucose load (2 g/kg body weight; a 200 g/l solution). Blood samples were drawn from the tail vein before (0 min) and 15, 30, 60, 90 and 120 minutes after the oral administration of glucose. The glucose level was determined with an Accu-Check Advantage blood glucose monitor (Roche Group, Indiana, USA). At the end of the experiment, blood samples were collected in heparinized tubes (2 U/μl) from the orbital venous plexus from 12-h-fasted rats and centrifuged at 4000 rpm

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1 To whom correspondence should be addressed. Tel: +91-184-2259128; Fax: +91-184-2250042; E-mail: yadavhariom@gmail.com
for 10 min. The plasma obtained was used to analyse the triglyceride level (by enzymatic method kit procured from Bayer Diagnostics, India) and insulin concentrations by using an enzyme immunoassay kit (Marcodia Pvt. Ltd., India) and insulin concentration. Glycosylated haemoglobin in the blood (measured with a cation-exchange resin kit (Monozyme Pvt. Ltd., India) and free fatty acids were measured by using an enzyme immunoassay kit (Marcodia from Bayer Diagnostics, India). Glycosylated haemoglobin in the blood was measured by using an enzyme immunoassay kit (Marcodia from Bayer Diagnostics, India) and insulin concentration. Glycosylated haemoglobin in the blood (measured with a cation-exchange resin kit (Monozyme Pvt. Ltd., India) and free fatty acids were measured by using an enzyme immunoassay kit (Marcodia from Bayer Diagnostics, India). Glycosylated haemoglobin in the blood (measured with a cation-exchange resin kit (Monozyme Pvt. Ltd., India) and free fatty acids were measured by using an enzyme immunoassay kit (Marcodia from Bayer Diagnostics, India). Glycosylated haemoglobin in the blood (measured with a cation-exchange resin kit (Monozyme Pvt. Ltd., India) and free fatty acids were measured by using an enzyme immunoassay kit (Marcodia from Bayer Diagnostics, India). Glycosylated haemoglobin in the blood (measured with a cation-exchange resin kit (Monozyme Pvt. Ltd., India) and free fatty acids were measured by using an enzyme immunoassay kit (Marcodia from Bayer Diagnostics, India). Glycosylated haemoglobin in the blood (measured with a cation-exchange resin kit (Monozyme Pvt. Ltd., India) and free fatty acids were measured by using an enzyme immunoassay kit (Marcodia from Bayer Diagnostics, India).

Table 2. Effect of Dahi Feeding on the Body Weight, Glycosylated Haemoglobin, Insulin, Triglyceride and Free Fatty Acids in Type 2 Diabetic Rats on the 42nd Day of the Experiment

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal</th>
<th>Control</th>
<th>Dahi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight gain (g)</td>
<td>98.43 ± 6.32</td>
<td>102.12 ± 4.32</td>
<td>95.11 ± 6.29</td>
</tr>
<tr>
<td>FBG (mmol/l)</td>
<td>6.39 ± 2.33</td>
<td>10.56 ± 2.12b</td>
<td>7.75 ± 1.01bh</td>
</tr>
<tr>
<td>Blood HaBc (%)</td>
<td>4.23 ± 1.32</td>
<td>7.98 ± 1.07b</td>
<td>5.87 ± 1.11bh</td>
</tr>
<tr>
<td>Plasma insulin (pmol/l)</td>
<td>210.11 ± 54.21</td>
<td>432.12 ± 67.34b</td>
<td>273.87 ± 54.98a</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>48.38 ± 3.23</td>
<td>75.23 ± 10.21b</td>
<td>52.12 ± 9.63a</td>
</tr>
<tr>
<td>Free fatty acids (mg/dl)</td>
<td>20.19 ± 3.21</td>
<td>42.11 ± 7.27b</td>
<td>21.29 ± 2.98NS</td>
</tr>
</tbody>
</table>

1Each value is the mean ± SD for 6 rats in each group. 2The dahi group differs significantly from the control and normal groups, respectively (p < 0.05).
3NS, No significant differences among the groups.

The gross chemical composition of dahi, viz. moisture (78.23%), total solid (21.08%), protein (3.94%), fat (2.52%) and lactose (3.73%), pH value (4.98) and titratable acidity (1.01%) were within the range typically found for normal dahi or yoghurt. As shown in Table 2, the body weight gain during the 6 weeks of the experimental period was not significantly different among the experimental animals of all three groups. The blood glucose level was significantly higher (39%) in the control group animals than in the normal group animals, whereas blood glucose was lower (17%) in the dahi-fed group animals. Similarly, the glycosylated haemoglobin level in the control and dahi-fed groups was significantly higher (46%, 27%, respectively) than that in the normal group. The blood glucose and glycosylated haemoglobin levels were significantly lower in the dahi-fed group (26% for both) than in the control group animals. Glycosylated haemoglobin is the product of a chemical reaction that takes place in blood with excess sugar under oxidative stress, and the molecule is stable over the life of the erythrocytes. The OGTT tests were performed at weekly intervals, although only the data for the 3rd and 6th week of the
OGTT of the control group animals was impaired by the 3rd week, whereas OGTT was impaired by the 4th week of the experimental period in the dahi-fed group of animals. The area under the curve after oral glucose loading ($AUC_{\text{glucose}}$) was significantly higher (39%) than that of the normal group of animals in the 3rd week of the experimental period, whereas the dahi-fed group of animals only showed slightly higher $AUC_{\text{glucose}}$ values (15%). However, after 6 weeks of the experimental period, the $AUC_{\text{glucose}}$ level was significantly higher (62%) in the dahi-fed group than in the control group of animals. The plasma insulin level in the dahi-fed group was significantly lower (36%) than in the control group of animals, but was higher (23%) than the normal animals. On the basis of these observations, we concluded that the dahi-supplemented diet might have prevented and/or delayed the increased blood glucose and impaired OGTT, and suppressed hyperinsulinemia during the experiment and may inhibit the development of the insulin resistance syndrome/type 2 diabetes induced by a high-fructose diet.

A limited number of studies have been conducted on the antidiabetic effect of lactic acid bacteria and their fermented products. Dahi used in the present study was made by the fermentation of Lactococcus cultures. These Lactococcus bacteria may have proteinase enzymes on the cell wall which degrade the casein protein of the milk during fermentation and might produce bioactive peptides; these may show the antidiabetic potential of dahi. The functional galacto-oligosaccharides (prebiotics) that were formed in this dahi during fermentation were also analysed by the HPLC method (data not shown here). Functional galacto-oligosaccharides have also been suggested to have a number of beneficial effects on human health which include bifidogenic activity, stimulation of mineral absorption, a hypolipidemic effect, and prevention of colon cancer. Lactic acid bacteria reaching the intestine can colonize on the intestinal epithelium of an animal which may reduce glucose absorption from the small intestine, although the mechanism is not known. The results presented here do not provide strong supportive evidence; we want to measure the in vitro glucose utilization by the bacterial culture, the glucose absorption by rats fed with dahi, and the active components of dahi.

Hypertriglyceridemia, hyperinsulinemia, hypercholesterolemia, hypertension and increased free fatty acids are common features in animal models of insulin resistance induced by a high-fructose diet. Increased levels of triglyceride and free fatty acids are the main predictors and/or causative agents for inducing the insulin resistance in type 2 diabetes. The triglyceride and free fatty acid levels were significantly higher (35% and 52%, respectively) in the control group animals than in the normal animals. In the dahi-fed group of animals, the triglyceride and free fatty acid levels were significantly lower (30% and 49%, respectively) than in the control group of animals. These results suggest that the dahi also suppressed the elevation of triglyceride and free fatty acids. However, the anti-hyperlipidemic effect of dahi needs further study.

The foregoing observations lead us to conclude that the dahi slightly inhibited the development of early hyperglycemia, glycosylated haemoglobin, hyperinsulinemia which might be causative factors for impaired glucose tolerance. The plasma insulin level was also higher (51%) in the control group than in the normal group of animals. The plasma insulin level in the dahi-fed group was significantly lower (36%) than in the control group of animals, but was higher (23%) than the normal animals. On the basis of these observations, we concluded that the dahi-supplemented diet might have prevented and/or delayed the increased blood glucose and impaired OGTT, and suppressed hyperinsulinemia during the experiment and may inhibit the development of the insulin resistance syndrome/type 2 diabetes induced by a high-fructose diet.

Fig. 1. Effect of the Dahi-Supplemented Diet on Oral Glucose Tolerance.
Rats (6–8 weeks old, male, $n = 6$) were administered 2 g/kg body weight of glucose after fasting for 12 h with a 15% dahi-supplemented high-fructose (21%) diet. The blood glucose level was measured from 0 to 120 min. Each value is the mean ± SD.
antidiabetic mechanism. Such a study should be conducted on the *Lactococcus* bacteria present in dahi individually as a culture (raw/lyophilized).

References