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# Oral administration of dahi containing probiotic *Lactobacillus acidophilus* and *Lactobacillus casei* delayed the progression of streptozotocin-induced diabetes in rats

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In this study, the effect of dahi containing probiotic *Lactobacillus acidophilus* NCDC14 and *Lactobacillus casei* NCDC19 ($\sim 73 \times 10^8$ cfu/g) on progression of streptozotocin (STZ)-induced diabetes in rats (15 g/day/rat) for 28 days was investigated. Feeding of probiotic dahi significantly suppressed the incremental peaks and area under the curve and delayed reduction of insulin secretion during oral glucose tolerance test more than skim milk or control dahi. The feeding of milk products reduced the total cholesterol, triglycerides, LDL and VLDL-cholesterol and increased HDL-cholesterol levels ($P<0{\cdot}05$). Moreover, probiotic dahi significantly suppressed STZ-induced oxidative damage in pancreatic tissues by inhibiting the lipid peroxidation and formation of nitric oxide, and preserving antioxidant pool such as glutathione content and activities of superoxide dismutase, catalase and glutathione peroxidase. The results suggest that the supplementation of probiotic *Lb. acidophilus* and *Lb. casei* with dahi cultures increased the efficacy of dahi to suppress STZ-induced diabetes in rats by inhibiting depletion of insulin as well as preserving diabetic dyslipidemia, and inhibiting lipid peroxidation and nitrite formation. This may empower antioxidant system of β-cells and may slow down the reduction of insulin and elevation of blood glucose levels.

**Keywords:** Diabetes, dahi, probiotics, antihyperlipidemic, antioxidant, *Lactobacillus*, milk, rat.

The consumption of functional foods with established antidiabetic components is perhaps one of the best documented strategies to treat and/or inhibiting the prevalence of diabetes, worldwide (Riezzo et al. 2005). Dairy foods constitute a family of natural functional foods with their established health benefits. Dahi is an Indian home made variant of yogurt, which differs from yoghurt in the use of mixed starters of mesophilic lactococci. The principle flavour inducing metabolite in dahi is diacetyl, which is relished more by people of South-Asian origin than the acetaldehyde flavour in yoghurt. Dahi has been considered as a functional food due to its several health benefits i.e. antidiarreal, anticarcinogenic, cholesterol lowering and antiatherogenic properties (Chawla & Kansal, 1984; Abbas & Jafri, 1992). Probiotics are the live microbial food supplements which give health benefits beyond basic nutrition upon consumption in sufficient amount (FAO/WHO, 2001). Different strains of lactic acid bacteria (LAB) such as *Lactobacillus* and *Bifidobacterium* are considered as important probiotics regimens. Fermented milk products such as yogurt, containing probiotic LAB, are well established for several health benefits (Reid et al. 2005). Few reports have dealt with the antidiabetic effect of LAB. Matsuzaki et al. (1997a, b), reported that oral administration of *Lb. casei* has a preventive effect on elevation of plasma glucose and reduction of plasma insulin levels by preventing immune mediated destruction of pancreatic β-cells in NOD and KK-Ay mice. Moreover, they also reported that feeding of *Lb. casei* supplemented diet strongly inhibited disappearance of β-cells and nitric oxide production in alloxan injected BALB/c mice (Matsuzaki et al. 1997c). Tabuchi et al. (2003) reported that oral administration of *Lb. rhamnosus* GG extensively delayed the induction of type 1 diabetes in STZ-injected rats. *Lb. acidophilus* NCDC14 and *Lb. casei* NCDC19 used in present study were selected for probiotic attributes i.e. acid and bile tolerance, colonization in intestinal tract,

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protoplast regeneration, surface hydrophobicity, antagonistic effect against pathogenic bacteria. From 50 lactobacilli strains (un-published data) these were chosen for preparation of probiotic dahi (PD), which has shown antidiabetic effect in type 2 diabetic rats (Yadav et al. 2007a). The purpose of present study was to investigate the suppressive effect of dahi supplemented with probiotic *Lb. acidophilus* and *Lb. casei* during the progression of STZ-induced diabetes in rats in comparison with skim milk (SM) and control dahi (CD) fed groups.

## Material and methods

### *Bacterial cultures and preparation of dahi*

*Lactococcus lactis* ssp *diacetylactis* NCDC60, *Lb. acidophilus* NCDC14 and *Lb. casei* NCDC19 were obtained from National Collection of Dairy Cultures, of the National Dairy Research Institute, Karnal, India. Raw buffalo milk was procured from the experimental dairy, of the institute. Milk was adjusted to 25 g fat/l by adding fresh SM and heated at 90 °C for 15 min, and cooled to 37 °C, aseptically. PD was prepared by inoculating this milk with $\sim 10^7$ cfu/ml *Lb. acidophilus*, $\sim 10^4$ cfu/ml *Lb. casei* and $\sim 10^6$ cfu/ml *Lc. lactis* (Yadav et al. 2005). CD was prepared by inoculating with 1% *Lc. Lactis* ($\sim 10^7$ cfu/ml). The final products contained bacterial counts of $\sim 73 \times 10^8$ cfu/g. The fat, protein, lactose, total solids and ash contents were ~0·45 to 2·5, 4·5, 3·27 to 4·18, 16 and 0·9%, respectively in milk and dahi samples, which were analysed by methods described in Association of Official Analytical Chemists (1995).

### *Animals and feeding schedule*

Male Wistar rats of 6–8 weeks old [156 ± 8·91 g body weight (b.w.)] were housed 3 animals per cage at 23–25 °C and 12/12 h light dark cycle in small animal house of the institute. Animals were divided into five groups ($n=6$ in each group) viz. (1) *normal group* (NCG); fed with standard chow (SC) of the small animal house, (2) *diabetic control group* (DCG); also fed with SC, (3) *SM fed group* (SMG); fed with SM with drinking water (15 ml/rat/day) and SC, (4) *CD fed group* (CDFG), fed with CD (15 g/rat/day) along with SC and (5) *PD fed group* (PDFG); fed with PD (15 g/rat/day) and SC. The SC contained 61% wheat, 28% chick pea, 1% casein, 5% refined groundnut oil, 4% salt mixture and 1% vitamin mixture. The salt and vitamin mixtures were prepared according to the American Official of Analytical Chemists (1995). Diabetes was induced by a single injection (i.p.) of 45 mg/kg b.w. of STZ (freshly prepared in 0·1 M-citrate buffer, pH 6·3) to overnight food deprived animals of DCG, SMG, CDFG and PDFG on d 0 of the experiment. However, NCG animals were injected with an equal volume of citrate buffer only. Food and water intake, urine volume and b.w. were analysed at weekly intervals by keeping animals in metabolic cages. Urine sugar, albumin and ketone bodies were determined by using Benedict's reagent, sulphosalicylic acid coagulation test and Rothera's reagent, respectively. The study was approved by the National Dairy Research Institute Animal Ethics Committee, and rats were maintained in accordance with the National Institute of Nutrition, India guidelines for the care and use of laboratory animals (Saionton, 2002).

### *Oral glucose tolerance test and insulin response test*

Oral glucose tolerance tests (OGTT) were performed at weekly interval throughout the experimental period. The rats were food deprived for 12 h before the administration of an oral glucose load (2 g/kg b.w.). Tail blood was drawn at 0, 30, 60, 90 and 120 min after glucose feeding. Glucose levels were determined with the help of Accu-Check Advantage Blood Glucose Monitor (Roche Group, Indiana, USA) by using one drop of whole blood and remaining blood sample was collected in heparinised (2 U/μl) vials and plasma was used for estimation of insulin levels by ELISA kit (Mercodia Diagnostics, Uppsala, Sweden). Area under the curve for glucose ($AUC_{glucose}$) was determined using the trapezoidal rule.

### *Blood and tissues collection*

At the last day of experiment, blood was collected in heparinized vials (2 U/μl) from overnight fasted animals by puncturing tail vein. Plasma was separated by centrifuging the blood samples at 5000 ***g*** for 10 min at 4 °C and used for analysis of blood lipids as described below. After blood collection, animals were killed by cervical dislocation and liver, pancreas, epididymal, retroperitoneal and abdominal fat, soleus and extensor digitorum longus (EDL) muscles were excised and weighed. The liver and pancreas were chopped in small pieces and stored at −70 °C till further use.

### *Biochemical Estimations*

Plasma total-cholesterol (Tc), triglyceride (TG) and HDL-cholesterol (HDLc) were estimated using enzymatic kits procured from Bayer Diagnostics India Ltd, Gujarat, India and LDL-cholesterol (LDLc), and VLDL-cholesterol (VLDLc) were calculated by using the Friedewald's equation (Friedewald et al. 1972). The hepatic tissue lipids were extracted according to Folch et al. (1957) and estimated as above. Liver glycogen content was determined by the method of Vander Varies (1954). Tissue lipid peroxidation as TBARS (Ohkawa et al. 1979), total nitrite (Moshage et al. 1995), GSH (Ellman, 1959), and the activities of catalase (Sinha, 1972), superoxide dismutase (SOD) (Marklund & Marklund, 1974), glutathione peroxidase (GPx) (Rotruck et al. 1973) and, total protein (Lowry et al. 1951) were determined according to the methods described previously.

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**Table 1.** Effect of SM, CD and PD supplemented diet feeding on various physiological parameters in normal and STZ-injected male Wistar rats

| Variable(s) | NCG | DCG | SMG | CDFG | PDFG |
|---|---|---|---|---|---|
| Initial body weight (g) | 187·12 ± 9·2[a] | 192·38 ± 8·2[a] | 189·2 ± 7·2[a] | 193·42 ± 7·3[a] | 191·32 ± 6·18[a] |
| Final Body weight (g) | 198·76 ± 5·3[a] | 185·97 ± 4·1[b] | 188·2 ± 2·8[b] | 193·22 ± 3·7[c] | 193·71 ± 4·91[c] |
| Body weight gain (g)* | 11·12 ± 4·15[a] | −6·39 ± 2·21[b] | −1·22 ± 0·4[c] | −0·20 ± 0·58[d] | 2·39 ± 1·83[e] |
| Food intake (g/d/rat)* | 19·23 ± 2·32[a] | 20·01 ± 3·38[a] | 18·29 ± 2·11[a] | 19·11 ± 3·22[a] | 19·89 ± 2·11[a] |
| Water intake (ml/d/rat)* | 82·28 ± 4·28[a] | 83·29 ± 4·83[a] | 82·11 ± 5·01[a] | 81·89 ± 5·77[a] | 82·01 ± 3·39[a] |
| Urine volume (ml/d/rat)* | 36·28 ± 2·99[a] | 35·78 ± 4·88[a] | 35·38 ± 5·13[a] | 36·76 ± 3·73[a] | 35·91 ± 2·99[a] |
| Liver weight† | 9·17 ± 2·56[a] | 10·25 ± 3·01[a] | 10·22 ± 1·10[a] | 10·20 ± 2·39[a] | 9·16 ± 1·33[a] |
| Pancreatic tissue† | 0·32 ± 0·05[a] | 0·31 ± 0·03[a] | 0·31 ± 0·04[a] | 0·32 ± 0·03[a] | 0·33 ± 0·08[a] |
| Epididymal fat† | 0·37 ± 0·04[a] | 0·30 ± 0·02[b] | 0·34 ± 0·04[b] | 0·38 ± 0·01[a] | 0·38 ± 0·02[a] |
| Retroperitoneal fat† | 0·23 ± 0·01[a] | 0·21 ± 0·03[a] | 0·21 ± 0·05[a] | 0·23 ± 0·02[a] | 0·24 ± 0·01[a] |
| Abodminal fat† | 1·56 ± 0·02[a] | 1·21 ± 0·04[b] | 1·28 ± 0·09[b] | 1·31 ± 0·08[b] | 1·41 ± 0·08[d] |
| Soleus muscle# | 45·21 ± 11·43[a] | 43·43 ± 13·20[a] | 43·65 ± 16·11[a] | 46·01 ± 14·82[a] | 45·33 ± 11·29[a] |
| EDL muscle# | 42·54 ± 3·32[a] | 40·54 ± 3·54[a] | 40·35 ± 4·10[a] | 43·53 ± 2·54[a] | 43·22 ± 2·99[a] |
| Urine sugar | −[a] | ++++[b] | ++++[b] | ++++[b] | ++++[b] |
| Urine albumin | −[a] | −[a] | −[a] | −[a] | −[a] |
| Urine ketone bodies | −[a] | −[a] | −[a] | −[a] | −[a] |

†# Values (mean ± SD of six animals in each group) were recorded as g/100 g and mg/100 g of body weight, respectively after 28 days and *were estimated during 28 day of the experimental period

[a–e] Values with common superscript in a row are not significantly different at the level of $P$ value <0·05

### *Statistical analysis*

Results were subjected to analysis of variance by using SPSS (SPSS Inc. Chicago) and significant differences among mean values of variables were determined by Benferroni's test. The values differed at the levels of $P<0·05$ were considered significant. Data were expressed as mean ± standard deviation (SD).

## Results

### *Dietary intake and physiological variables of animals*

After STZ-injection, the b.w. of rats was significantly decreased during the 28 d experimental period. The rate of reduction was highest in CDG and lowest in PDFG animals among all the STZ-injected groups (Table 1). The type of diet did not significantly affect the intake of food and water, as well as excretion of urine volume. The liver and pancreatic organ weights were not statistically different in all the groups. Weights of epididymal fat were significantly lower in DCG, SMG and CDFG ($P<0·05$) while these measures were closer to the normal in CDFG and PDFG. Similarly, abdominal fat pad was also higher in PDFG among all the STZ-injected rats ($P<0·05$). No significant changes were observed in retroperitoneal fat content among all the groups. Moreover, no significant differences were observed in the weight of soleus and EDL muscles in all groups. During last week of experiment, urine sugar was significantly increased and appeared as 4+ (>2 gm/dl) in all the STZ-injected rats (Table 1), while urine protein and ketone bodies were not detected in any group of the animals.

### *Effect on OGTT and insulin response*

The OGTT showed a significant decrease of insulin response in DCG, SMG and CDFG after 7 d (data not shown). However, feeding of PD maintained OGTT as normal on d 7 but tolerance was reduced 14 d after the STZ-injection. At the end of study (28 d), the $AUC_{glucose}$ values were lowest in PDFG among treated groups, followed by CDG<SMG<DCFG. In contrast, the $AUC_{insulin}$ values were highest in PDFG among all the STZ-injected animals (Fig. 1).

### *Effects on liver glycogen content*

As shown in Table 2, the liver glycogen was lowest in DCG among all the groups. The feeding of PD maintained the liver glycogen near to the normal values in STZ-injected rats. The oral administration of SM and CD also inhibited the reduction of glycogen accumulation in liver but the effect of SM was lower than CD ($P<0·05$).

### *Effects on blood and liver lipid content*

Table 2 also depicts that the plasma Tc, TG, LDLc, VLDLc, LDLc/HDLc ratio were highest and HDLc was lowest in the DCG among all the groups after 28 d ($P<0·05$). However these variables were maintained near to the normal in PDFG. Similarly, liver Tc and TG also highest in DCG while maintained as normal by feeding of PD. However feeding SM or CD was moderately (or partially) effective in mitigating the effect of STZ-induced diabetes on plasma and liver lipid profiles.

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**Table 2.** Effect of SM, CD and PD supplemented diet feeding on hepatic glycogen, plasma and tissue lipids in normal and STZ-injected male Wistar rats after 28 days of the experimental period

| Variable(s) | NCG | DCG | SMG | CDFG | PDFG |
|---|---|---|---|---|---|
| Liver glycogen content (mg/g tissue) | 7·94 ± 0·21[a] | 4·11 ± 0·08[b] | 4·32 ± 0·13[c] | 4·35 ± 0·22[c] | 5·01 ± 0·19[d] |
| Plasma total cholesterol (mg/dl) | 67·3 ± 5·2[a] | 98·20 ± 9·58[b] | 83·3 ± 5·59[c] | 82·39 ± 4·23[c] | 71·58 ± 3·01[a] |
| Plasma triglyceride (mg/dl) | 53·29 ± 1·49[a] | 59·39 ± 1·20[b] | 59·02 ± 1·12[b] | 58·98 ± 1·29[b] | 54·39 ± 1·01[a] |
| Plasma HDL-cholesterol (mg/dl) | 34·39 ± 0·99[a] | 28·39 ± 1·09[b] | 31·78 ± 1·13[a] | 31·38 ± 1·08[a] | 33·89 ± 1·11[a] |
| Plasma LDL-cholesterol (mg/dl) | 22·26 ± 2·32[a] | 57·94 ± 3·19[b] | 38·72 ± 2·72[c] | 39·22 ± 3·65[c] | 26·81 ± 3·24[d] |
| Plasma VLDL-cholesterol (mg/dl) | 10·65 ± 0·68[a] | 11·87 ± 0·64[a] | 11·80 ± 0·42[a] | 11·79 ± 1·86[a] | 10·88 ± 1·60[a] |
| LDL/HDL ratio | 0·64 ± 0·09[a] | 2·04 ± 0·12[b] | 1·22 ± 0·11[c] | 1·24 ± 0·19[c] | 0·79 ± 0·06[a] |
| Liver total cholesterol (mg/g tissue) | 2·93 ± 0·39[a] | 4·35 ± 0·85[b] | 3·49 ± 0·58[c] | 3·48 ± 0·53[c] | 2·58 ± 0·53[a] |
| Liver triglyceride (mg/g tissue) | 10·43 ± 1·22[a] | 18·29 ± 0·91[b] | 13·39 ± 1·59[c] | 13·29 ± 1·03[c] | 10·49 ± 1·01[a] |

[a–d] The values (mean ± SD) with common superscript in a row are not significantly different ($P<0·05$)

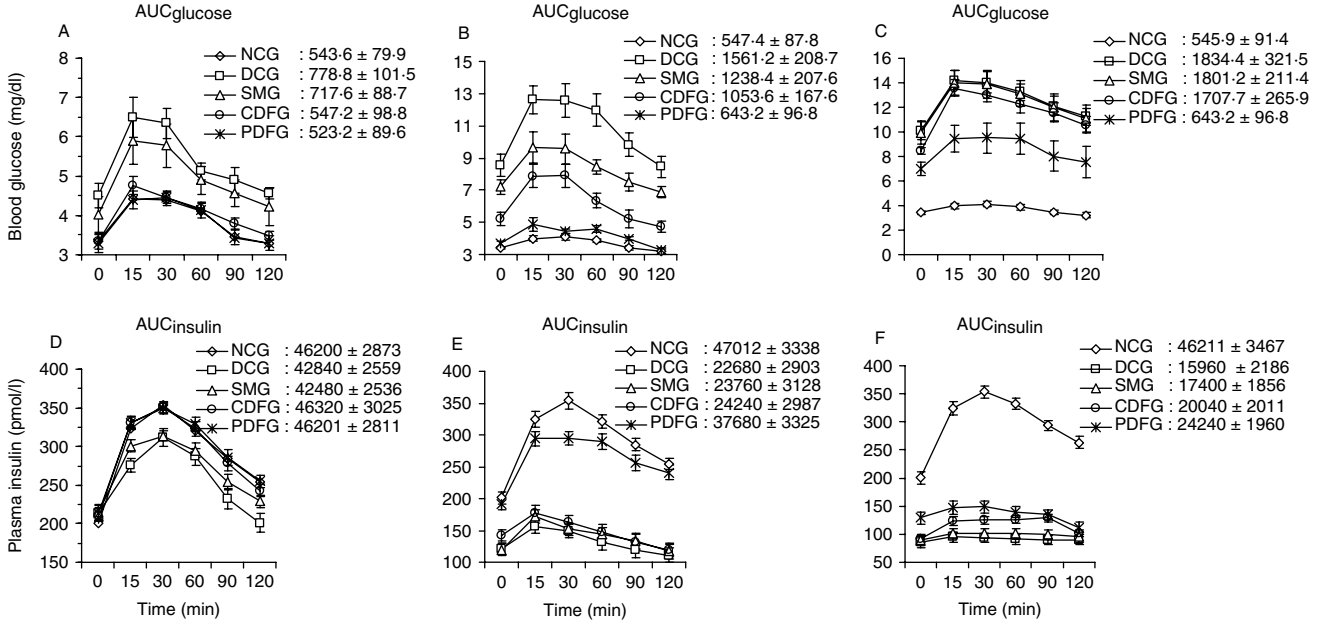


**Fig. 1.** The effect of feeding of probiotic dahi on induction of glucose intolerance by increasing blood glucose and reducing plasma insulin in STZ-injected rats in comparison to normal, diabetic control, skim milk and control dahi-fed group of animals during 28 days. Glucose solution (2g/kg b.w.) was orally administered in overnight fasted animals and blood glucose (A, B, C) and plasma insulin (D, E, F) were estimated as described in text at each week of experimental period but here the graphs of 14 (A and D), 21 (B and E) and 28 (C and F) days were represented.

*Effects on oxidative stress measures*

Twenty eight d after STZ-injection, the TBARS and total nitrite were drastically increased in the pancreatic tissues of DCG compared with NCG (Table 3). The feeding of PD arrested the elevation of these factors which remained significantly lower after 28 d than in other diabetic animals ($P<0·05$). Moreover, GSH and enzymatic activities of catalase, SOD and GPx were significantly depleted in pancreatic tissues of DCG compared with NCG ($P<0·05$). The oral administration of PD also arrested the depletion of GSH and enzymatic activities of catalase, SOD and GPx in STZ-injected rats, while, SM and CD have moderate activities for these effects.

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**Table 3.** Effect of SM, CD and PD supplemented diet feeding on oxidative stress measures in normal and STZ-injected male Wistar rats after 28 days of the experimental period

| Variable(s) | NCG | DCG | SMG | CDFG | PDFG |
|---|---|---|---|---|---|
| TBARS (nmol/g tissue) | $23{\cdot}20 \pm 2{\cdot}49^{a}$ | $102{\cdot}4 \pm 3{\cdot}95^{b}$ | $92{\cdot}39 \pm 2{\cdot}89^{c}$ | $90{\cdot}38 \pm 3{\cdot}48^{c}$ | $71{\cdot}37 \pm 2{\cdot}89^{d}$ |
| Nitrite (nmol/l) | $1{\cdot}28 \pm 0{\cdot}12^{a}$ | $6{\cdot}49 \pm 0{\cdot}91^{b}$ | $5{\cdot}09 \pm 0{\cdot}51^{b}$ | $4{\cdot}89 \pm 0{\cdot}83^{b}$ | $3{\cdot}49 \pm 0{\cdot}74^{c}$ |
| Reduced glutathione (mg/g tissue) | $126{\cdot}39 \pm 4{\cdot}83^{a}$ | $105{\cdot}28 \pm 2{\cdot}49^{b}$ | $107{\cdot}38 \pm 4{\cdot}38^{b}$ | $108{\cdot}38 \pm 3{\cdot}48^{b}$ | $119{\cdot}27 \pm 2{\cdot}11^{c}$ |
| Catalase (U/mg protein) | $17{\cdot}38 \pm 0{\cdot}67^{a}$ | $8{\cdot}58 \pm 0{\cdot}54^{b}$ | $8{\cdot}47 \pm 0{\cdot}39^{b}$ | $10{\cdot}18 \pm 0{\cdot}59^{c}$ | $14{\cdot}48 \pm 0{\cdot}47^{d}$ |
| Superoxide dismutase (U/mg protein) | $13{\cdot}02 \pm 0{\cdot}11^{a}$ | $5{\cdot}30 \pm 0{\cdot}30^{b}$ | $8{\cdot}29 \pm 0{\cdot}21^{c}$ | $9{\cdot}01 \pm 0{\cdot}38^{c}$ | $11{\cdot}10 \pm 0{\cdot}21^{d}$ |
| Glutathione peroxidase (U/mg protein) | $5{\cdot}43 \pm 0{\cdot}89^{a}$ | $3{\cdot}19 \pm 0{\cdot}11^{b}$ | $3{\cdot}29 \pm 0{\cdot}10^{b}$ | $3{\cdot}89 \pm 0{\cdot}21^{b}$ | $4{\cdot}69 \pm 0{\cdot}27^{c}$ |

[a–d] The values (mean ± SD) with common superscript in a row are not significantly different ($P < 0{\cdot}05$)

## Discussion

In present study, the effect of oral administration of dahi containing probiotic *Lb. acidophilus* and *Lb. casei* along with dahi culture on progression of STZ-induced diabetes in rats was evaluated. It was found that PD was more effective in suppression of the progression of STZ-induced diabetes and its complications such as dyslipidemia and oxidative stress compared with the SM and CD. It is well known that STZ selectively kills insulin secreting pancreatic β-cells resulting in a decrease in endogenous insulin release, which paves the ways to diminished utilization of glucose by tissues (Wilson et al. 1984). In type 1 diabetes β-cells are destroyed by autoimmune reactions, also long term type 2 diabetes leads to β-cell damage by various unknown mechanisms (Robertson & Harmon, 2006). In present study, the feeding of PD significantly delayed the progression of STZ-induced diabetes by suppressing the elevation of glucose intolerance and blood glucose as well as reduction of insulin levels during 28 d of the experiment. It indicates that PD may delay the STZ-induced alteration in glucose homeostasis by maintaining insulin levels which might be due to protection of pancreatic β-cell from damage. In addition, Fig. 1 indicates that the levels of insulin were not altered 14 d after STZ-injection among all groups of animals unlike the increased blood glucose levels, the reason behind these results may be the allied production of non-function insulin by STZ-exposed β-cells. PD was unable to fully suppress the induction of STZ-induced diabetes in rats after 28 d, this might be due to the chronic effect of STZ on β-cells and suppressive effect of PD was overcome in a time dependent manner in STZ-injected rats.

Insulin stimulates the glycogen synthesis in liver tissues in the presence of higher levels of glucose in blood (Klover & Mooney, 2004). Depleted insulin levels in STZ-induced diabetes may results in reduction of liver glycogen content that might be due to slow down of the insulin activated glycogenesis pathway. A similar trend was also observed in the present study. The oral administration of PD maintained liver glycogen content near normal; demonstrating that PD has the ability to attenuate insulin depletion sufficiently to stimulate glycogen synthesis in liver tissues till 28 d.

Feeding of PD arrested the elevation of Tc and TG and depletion of HDLc in blood as well as liver which suggests that PD feeding may delay the progression of diabetes induced atherosclerosis [a major causative agent for induction of the foremost diabetic complication (Henry, 2001)]. Several workers also reported, the cholesterol lowering activity of milk and its fermented products containing LAB (Taylor & Williams, 1998). It has been proposed that various milk components such as orotic acid, pyrimidine like nucleotide, calcium or hydroxyl methyl glutaric acid attenuate the de-novo synthesis of cholesterol through inhibition of NADPH formation (a reducing power required for biosynthesis of cholesterol) HMG-CoA synthase (a rate limiting step in cholesterol biosynthesis) in liver and increase cholesterol clearance from blood stream (by enhancing excretion of bile acids) (Agrawal & Kansal, 1991). In addition, fermentation of milk with LAB increases bioavailability of these components and increased absorption in gastrointestinal tract allows increased function (Roberfroid, 2000).

Oxidative stress is a hallmark of STZ-induced β-cell damage. STZ destroys β-cells increasing production of superoxide radical, $H_2O_2$ and hydroxyl radicals (Szkudelski, 2001). The most commonly used indicator of lipid peroxidation is TBARS (Lyons, 1991). In the present study, TBARS levels in pancreatic tissues were drastically increased in DCG after STZ administration. However, it was significantly lower in PDFG, indicated that the formation of TBARS was suppressed by PD feeding which may be due to inhibition of free radical formation and insulin reduction. Furthermore, nitric oxide (NO) has been demonstrated to be an effector molecule responsible for pancreatic β-cells destruction. STZ liberates toxic amounts of NO by increasing NO synthase activity that inhibits the activity of aconitase and participates in DNA damage. As a result of this, β-cells undergo destruction by necrosis (Szkudelski, 2001). The oral administration of PD also diminished the elevation of total nitrite in pancreatic tissues and thus might be preventing β-cell destruction. A biodefensive antioxidant system protects tissue from free radical damage. Diabetes reduces the antioxidant capacity of tissues which increases deleterious effects of free radicals. A sulphydryl tripeptide; glutathione is known to protect the cellular system against toxic effects of lipid

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peroxidation (Ahmed, 2005). During diabetic condition and increased oxidative stress, the GSH content decreases due to either higher utilization rate such as through Gpx activity and/or lower production (Anuradha & Selvam, 1993). Gpx is an enzyme that catalyzes the decomposition of $H_2O_2$ and other organic hydroperoxide to non-toxic products at the expense of the GSH (Bruce et al. 1982). Its activity may be reduced by radical induced inactivation and/or glycation of enzyme (Rajasekaran et al. 2005). The decreased GPx activity may allow accumulation of toxic products. In the present study, feeding of PD to STZ-injected rats significantly inhibited the decline of GSH content suggesting that it either enhanced biosynthesis of GSH or reducing the oxidative stress leading to less degradation of GSH or both. Moreover, fermented milk products containing several antioxidant sulphydryl group components may also increase the substrate pool for GSH biosynthesis (Aneja et al. 2002). SOD and catalase are two antioxidant enzymes which sequentially detoxify oxygen free radicals viz. SOD scavenges superoxide radical to $H_2O_2$ and molecular oxygen (McCord et al. 1976) and $H_2O_2$ is further detoxified by catalase and converted into water and molecular oxygen (Chance et al. 1952). In the present study, the activities of SOD and catalase were maintained near to normal by feeding of PD, which indicates that PD diminished the formation of superoxide anions and $H_2O_2$ (substrates of these enzymes).

As observed above, the PD was additionally effective in suppression of STZ-induced consequences in carbohydrates and lipid metabolism as well as oxidative stress compared with SM and CD. This may be due to higher availability of biologically active substances in PD than CD, i.e. bioactive peptides, organic acids (un-published data), free fatty acids, conjugated linoleinic acid and complex oligosaccharides (Yadav et al. 2007b, c).

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