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# Therapeutic effects of probiotic fermented milk (LGG and *L. casei* NCDC 19) on progression of type 2 diabetes

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**Abstract:** In today's world where diabetes has become an epidemic, our aim evaluated the effect of probiotics fermented milk prepared using *Lactobacillus rhamnosus* GG (LGG) and *Lactobacillus casei* NCDC19 on progression of type 2 diabetes. Male albino Wistar rats were fed with normal chow diet (control) and HFD i.e. high fructose diet (68.35% fructose) for 63 days. The high fructose diet fed rats were also given LGG and *L. casei* NCDC 19 fermented milk along with HFD till the end of study. The groups fed with probiotic fermented milk along with HFD showed low fasting blood glucose, glycosylated haemoglobin, cholesterol, triglycerides than the pure diabetic group fed with only HFD. Oxidative stress was also decreased in these groups. These results concluded that probiotic fermented milks have beneficial approach against type 2 diabetes. Therefore continuous feeding of fermented milks can be considered as an important dietary defense weapon to fight against type-2 diabetes in our daily life.

**Keywords:** Type 2 diabetes, Fermented milk, LGG, *L. casei* NCDC19, Fructose diet, probiotic

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Rapidly increasing global prevalence of Diabetes Mellitus, a significant cause of concern has made the world to sit on a time bomb which is ticking very fast. The incidences of diabetes are snowballing at such a high rate that this disease has changed its status from an endemic to global epidemic. Diabetes mellitus type 2 or non-insulin-dependent diabetes mellitus traditionally considered as a disease of adults is the major driver i.e. the most common form of diabetes (95%). As it is a heterogeneous disorder, it is manifested by high blood sugar level often associated with obesity, hypertension, elevated cholesterol (combined hyperlipidemia) and with some complications, the condition is often termed as Metabolic Syndrome (Basciano et al. 2005; Singh et al. 2014). Type 2 diabetes involves 2 fundamental abnormalities: resistance to the biological activities of insulin in glucose and lipid metabolism and inadequate insulin secretion from pancreatic β-cells (Goldstein, 2003).

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According to WHO (2009), type 2 diabetes is preventable and the solution lies in our dietary modifications mainly. Probiotics the live microorganisms which confer the health benefit to the host were first known in 1965 when Lilly and Stillwell first used it to describe it as any substance or organism that contributes to intestinal microbial balance, and Fuller in 1989 further emphasized their role in health. Historically, CHARAKA SAMHITA, a treatise on Ayurvedic medicine (written in 1000 BC), has explained the beneficial microbial flora of the gastrointestinal tract as "Jataragni" (fire in the stomach) which acts as a sustaining force for all living beings and "Takra," *i.e.*, fermented milk, as "Amrita" or elixir. Now it has become a well-established fact that the *Lactobacillus* strain has the ability to stabilize the healthy intestinal flora and destroys the pathogenic strains present therein. Elie Metchnikoff in 1907 was the first to propose a scientific rationale for the role of *Lactobacilli* in maintaining health and longevity one hundred years ago.

Probiotics have been used for centuries in the manufacture of cultured dairy products. Probiotics are widely consumed in the form of fermented milk which is the most simpler and excellent vehicle way for delivering probiotics in the body. Milk ferments as a

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panacea have been documented in the therapeutic treatment of a variety of disorders e.g. amelioration of lactose intolerance due to reduced cholesterol level, against various human alimentary tract diseases i.e. colitis (Rasic et al. 1978; Davis et al. 1975), constipation (Davis et al. 1975; Seneca, 1975), diarrhoea (DeDios et al. 1978), gastroenteritis (Davis et al. 1975), indigestion (Tatochenko, 1952), intoxication (Galvan et al. 1944), stomatitis (Steyn, 1969), hypercholesteremia (Mann et al. 1974), kidney and bladder disorders (Rasic et al. 1978), liver and bile disorders (Seneca, 1975; Finzi, 1967), obesity (Rasic et al. 1978), osteoporosis (Seneca, 1957), tuberculosis (Finzi, 1967; Kosikowski, 1966), cancer (Reddy, 1983; Shahani, 1983; Shackleford, 1983), calcium deficiency (Depuis, 1964). There are also reports claiming that functional foods have anti-diabetic effect (Hill et al. 2002). Yadav et al. (2007) also studied the effect of probiotic dahi culture in high fructose fed rats. However, evidence supporting the antidiabetic effects for fermented milk products is still lacking.

*Lactobacillus rhamnosus GG* which is nature's most potent probiotic has been well documented against treatment and prevention of acute diarrhoea in children (Guandalini et al. 2000; Shornikova et al. 1997; Pant et al. 1997), prevention and treatment of allergies (Kalliomaki et al. 2001; Rautava et al. 2002; Kalliomaki et al. 2003; Isolauri et al. 2000) prevention of traveller's diarrhoea (Oksanen et al. 1990; Hilton et al. 1997) treatment of rheumatoid arthritis (Baharav et al. 2004; Hatakka, 2003) and irritable bowel syndrome (O'Sullivan et al. 2000), prevention of cancer (Goldin, 1996; Lim et al. 2002; Lahtinen et al. 2004; Haskard, 2000) prevention of complications of liver disease (Nanji et al. 2005; Bauer et al. 2002) and various other diseases. LGG was also studied for its antidiabetic effect in streptozotocin-induced diabetic rats by Tabuchi et al in 2003. However further documentation to support such an effect is needed. In the present study we sought to determine the antidiabetic and its associated metabolic disorders e.g. antioxidative and hypercholesteromic effect of *Lactobacillus rhamnosus GG*. LGG was obtained from the symbiotic lab of dairy microbiology division of National Dairy Research Institute, Karnal who in turn got it as a gift from Proff. Goldin and Gorbach. We also selected another studied probiotic culture *L. casei NCDC 19* which we got from National Collection of Dairy Cultures (NCDC), N.D.R.I. Karnal along with LGG. Under this we studied the effect of feeding of LGG fermented milk and *L. casei NCDC 19* fermented milk on progression of type 2 diabetes in animal model.

## MATERIALS AND METHODS

### Animals

A total of 32 male albino rats of Wistar strain used in this study were obtained from National Institute of Pharmaceutical Research, Mohali at approximately 6-7 weeks of age. Their initial body weights were not significantly different (125-150 gm). This experimental protocol was approved by the National Dairy Research Institute Animal Ethics Committee, and rats were maintained in accordance with the National Institute of Nutrition, guidelines for the care and use of laboratory animals. All animals were housed 4 per cage in stainless steel wire cages at an ambient temperature of 21-25$^0$C, relative humidity (50-60%) and maintained on a reverse 12-hour dark (9 AM to 9 PM) and light (9 PM to 9 AM) cycle.

### Diets and experiment protocol

Initially all animals were fed with pellet diet and maintained on this diet for 1 week until they acclimatize. Food and water were given *ad libitum*. The rats were then randomly divided into 5 groups of 8 rats each. The following experimental groups were then maintained as follows:

**Group 1** Normal Control group received the control diet i.e. AIN-93 G (Reeves, 1993) and tap water *ad libitum*

**Group 2** Diabetic control group received the fructose enriched diet (68.35% fructose) and tap water *ad libitum*

**Group 3** Skim milk fed group received skim milk powder supplemented high fructose diet and tap water *ad libitum*

**Group 4** LGG fermented milk fed group received LGG fermented milk supplemented high fructose diet and tap water *ad libitum*

**Group 5** *L.casei NCDC 19* fermented milk fed group received *L. casei* NCDC 19 fermented milk supplemented high fructose diet and tap water *ad libitum.*

The composition of diets is given in Table 1. All animals were fed the experimental diets for 63 days.

### Blood and tissue collection

At the end of experimentation period after 63 days, all rats were dissected by cervical dislocation after 12-h-overnight fasting. Blood was collected using sterilized syringes from their heart in tubes containing ethylenediaminetetraacetate (EDTA) (2U/µL). 50 µl whole blood samples was used to determine the fasting blood glucose (FBG) and glycosylated haemoglobin (HbA1c) and remaining samples were centrifuged at 5000 × g for 10 min. at 4$^0$C to separate the plasma for triglycerides and cholesterol estimation. Immediately after sacrifice, abdomen was open by midline incision, liver was quickly excised, washed with chilled 0.2 M phosphate buffer saline (PBS; pH 7.2), frozen in liquid nitrogen, and stored at -70$^0$C. Later these liver samples were analysed for the oxidative stress.

**Table 1** Composition (g/Kg) of standard diet of normal control group (NCG), diabetic control group (DCG), skim milk fed group (SMG), *LGG* fermented milk group (LGGSM), *L. casei* NCDC 19 fermented milk group (LCSM) given to the rats

| Constituent | NCG | DCG | SMG | LGGSM | LCSM |
|---|---|---|---|---|---|
| Starch | 584.5 | 24.5 | - | - | - |
| Casein | 200.0 | 200.0 | 74.5 | 74.5 | 74.5 |
| Sucrose | 100.0 | - | - | - | - |
| Soybean oil | 70.0 | 70.0 | 70.0 | 70.0 | 70.0 |
| Mineral mixture* | 30.5 | 30.5 | 30.5 | 30.5 | 30.5 |
| Vitamin mixture* | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 |
| Choline chloride | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| Methionine | 3.0 | 3.0 | 3.0 | 3.0 | 3.0 |
| Fructose | - | 660.0 | 660.0 | 660.0 | 660.0 |
| Skim milk powder | - | - | 150 | - | - |
| Fermented milk | - | - | - | 150 | 150 |

*Vitamin and mineral mixture prepared and mixed according to AOAC, 1990

**Analytical measurement**

The blood glucose was monitored using glucometer (All Medicus Co. Ltd., Gyeonggi, Korea). Glycosylated hemoglobin was measured by using commercial kit from Monozyme Pvt. Ltd., Secunderabad, India. Level of triglycerides and cholesterol were analyzed in plasma using kits from Biovision, Inc, USA respectively. The oxidative stress [i.e. activities of catalase, super oxide dismutase (SOD) and glutathione peroxidase (GPx)] was estimated in homogenized liver (Cohen et al. 1970) collected from experimental animals. Activity of catalase was estimated spectrophotometrically using the method of Aebi, 1984. For super oxide dismutase (SOD), method of Marklund and Marklund (1974) was used whereas glutathione peroxidase (GPx) was assayed by the method of Lawrence and Burk (1976).

**Statistical analysis**

All data sets were represented as mean ± standard error of mean (SEM). Comparisons of findings between groups were made via statistical analysis of data sets using one-way analysis of variance (ANOVA) with Tukey's studentized range (HSD) test. A P-value of <0.05 was considered as statistically significant.

**RESULTS**

**Effect on fasting blood glucose and glycosylated hemoglobin**

Table 2 shows the effects of probiotic fermented milks on fasting blood glucose and glycosylated hemoglobin. Diabetes has a direct impact on the blood glucose level. As expected the fasting blood glucose levels started increasing in all the groups as compared to normal control group and recorded the highest levels in DCG group after 63 days. This increase in blood glucose was significantly very less (P <0.05) in both the fermented milk-fed groups i.e. LGGSM and LCSM than DCG because they both were also fed with their respective fermented milk diets along with HFD which was contributing to lowering of blood glucose. Skim milk fed group did not show any significant lowering in blood glucose. Glycosylated hemoglobin ($HBA_{1C}$) was recorded highest in DCG group rats (Table 2) among all the groups of animals during progression of diabetes after 9 weeks. These values also increased in all other groups along except NCG, but this increase was significantly lowest in LGGSM (P <0.05). Skim milk fed group did not show any significant effect in both the cases.

**Table 2** Effect of different experimental diets on fasting blood glucose (FBG) and glycosylated haemoglobin ($HBA_{1C}$) after 63 days

| | Parameters | |
|---|---|---|
| | FBG (mg/dl) | $HBA_{1C}$ (%) |
| NCG | 105.36±4.9$^{a}$ | 4.64±0.03$^{a}$ |
| DCG | 244.68±11.5$^{c}$ | 6.95±0.01$^{d}$ |
| SMG | 237.64±6.1$^{c}$ | 6.67±0.03$^{d}$ |
| LGGSM | 166.47±6.2$^{b}$ | 6.28±0.02$^{b}$ |
| LCSM | 186.34±6.3$^{b}$ | 6.43±0.01$^{c}$ |

$^{a,b,c,d}$ Values (means ± SEM; n = 8) with different superscript in a column are significantly different at the level of P <0.05

**Effect on plasma lipid profile**

Cholesterol values (Table 3) highest in DCG (119.06 mg/dl) when measured at the end of experimental period were also found to increase in all other groups but to a lesser extent. Cholesterol values were decreased in LGG and *L. casei* fermented milk treated groups showing the effect LGG and *L. casei* NCDC 19 fermented milk. In case of triglycerides, as expected with time the triglycerides level was increased in all the groups, highest in DCG when measured after 63 days. However this increasing trend was significantly less (P <0.05) in LGGSM and LCSM groups fed with LGG and *L. casei* NCDC 19 fermented milk product. LGG fermented milk was found most effective in controlling the level of cholesterol and triglycerides.

**Effect on oxidative stress in liver**

**Table 3** Effect of different experimental diets on plasma cholesterol and triglycerides after 63 days

| | Parameters | |
|---|---|---|
| | **Cholesterol (mg/dl)** | **Triglycerides (mg/dl)** |
| NCG | 80.17±3.4$^{a}$ | 58.06±4.4$^{a}$ |
| DCG | 119.06 ± 5.2$^{c}$ | 129.66±2.2$^{f}$ |
| SMG | 116.24±8.3$^{bc}$ | 127.22±2.8$^{ef}$ |
| LGGSM | 98.96±4.6$^{bca}$ | 114.39±3.5$^{ed}$ |
| LCSM | 103.82±6.9$^{bca}$ | 121.74±3.9$^{efd}$ |

$^{a,b,c,d,e,f}$ Values (means ± SEM; n = 8) with different superscript in a column are significantly different at the level of P <0.05

The results expressed in this Table 4 indicate the effect of different experimental diets on oxidative stress in liver (catalase, superoxide dismutase and glutathione peroxidase). At the end of study, it was found that catalase content decreased in liver of DCG animals to a highly significant extent. However, the feeding of LGG and *L. casei* NCDC 19 fermented milk produced an effect of elevation in catalase content and almost to the similar extent (LGGSM and LCSM group) (P<0.05). Similar results were produced during analyses of superoxide dismutase activity i.e. (LGGSM and LCSM) group animals showed the highest and similar increase in enzyme activity. However LGGSM group animals showed better increase in activity of glutathione peroxidase as compared to LCSM.

## DISCUSSION

Administration of fructose rich diet (68.35%) for a long period induces complications related to type 2 diabetes e.g. high blood glucose, glycosylated hemoglobin, cholesterol, triglycerides and oxidative stress (Vasdev et al. 2004; Thirunavukkarasu, 2004; Patel et al, 2010). Keeping in view the increasing prevalence of diabetes worldwide and considering it as a major ill factor of the population, the present study was an attempt to evaluate the therapeutic effect of probiotic fermented milks containing LGG and *L. casei* NCDC 19 during progression of type 2 diabetes. Continuous feeding of HFD for 63 days gave severe hyperglycemia (>240 mg/dL fasting blood glucose) (Table 2). Feeding of LGG and *L. casei* NCDC 19 fermented milks along with HFD exhibited significant lowering of fasting blood glucose levels in these animals.

These results were consistent with the study of antidiabetic potential of probiotic dahi done by Yadav et al. 2007. Antidiabetic dahi also showed lowering of hyperglycemia like the LGG and *L. casei* NCDC 19 fermented milk. However LGG fermented milk was more effective in lowering (23.45%) of glycosylated hemoglobin when compared to diabetic control group at the end of study. Plasma lipid profile was also improved in LGGSM and LCSM groups by lowering of cholesterol and triglycerides significantly. LGG and *L. casei* NCDC 19 were equally effective in lowering the level of cholesterol but LGG was reported to decrease triglycerides more than *L. casei* during this study. Various researchers have also explored the hypolipidemic effects of fermented milk products/ probiotics enriched foods in different dietary models such as high cholesterol diet fed rats (Zommara, 1998; Chawla and Kansal, 1984, Yadav, 2007). Anderson et al. explored the effect of fermented milk containing *L. acidophilus* L1 on serum cholesterol and triglycerides in hyperlipidemic humans. Daily consumption of 200 g of yogurt containing *L. acidophilus* L1 after each dinner contributed to a significant ($P$ < 0.05) reduction in serum cholesterol concentration (-2.4%) compared to the placebo group. Probiotic fermented milks also act as an effective anti-oxidant as they caused the elevation of catalase, SOD and glutathione peroxidase which are the key enzymes related to anti-oxidative stress. LGG and *L. casei* NCDC 19 are equally effective in improving the activities of catalase and SOD. Zhang et al. 2010 studied *L. casei* and found that it might help to alleviate oxidative stress, as they appeared to reduce lipid peroxidation and improved lipid metabolism both in blood and liver with hyperlipidemia in vivo. The effect of *Lactobacillus rhamnosus* IMC 501® and *Lactobacillus paracasei* IMC 502® on oxidative stress in athletes was studied by Daniele et al. 2011 during a four-week period of intense physical activity. They proved that the two strains exert strong antioxidant activity. Athletes and all those exposed to oxidative stress may benefit from the ability of these probiotics to increase antioxidative enzyme activities levels and neutralize the effects of reactive oxygen

**Table 4** Effect of different experimental diets on oxidative stress in liver after 63 days

| **Parameters** | **NCG** | **DCG** | **SMG** | **LGGSM** | **LCSM** |
|---|---|---|---|---|---|
| **Catalase** (U/mg protein) | 72.24±1.69$^{a}$ | 19.14±1.00$^{e}$ | 21.05±1.21$^{ed}$ | 41.64±0.95$^{c}$ | 40.08±1.59$^{c}$ |
| **SOD** (U/mg protein) | 45.04±1.69$^{a}$ | 21.73±2.35$^{d}$ | 22.78±2.02$^{d}$ | 29.11±1.00$^{cbd}$ | 28.29±1.18$^{cbd}$ |
| **GPx** (U/mg protein) | 49.62±1.35$^{a}$ | 26.66±0.60$^{e}$ | 30.13±1.43$^{de}$ | 39.34±2.04$^{bc}$ | 36.53±1.66$^{dc}$ |

$^{a,b,c,d,e}$ Values (means ± SEM; n = 8) with different superscript in a column are significantly different at the level of P <0.05

species.

**Conclusion**

In conclusion, the present study has shown that ingestion of probiotic fermented milks containing LGG and *L. casei* NCDC 19 significantly suppress various risk factors of type 2 diabetes i.e. fasting blood glucose, glycosylated hemoglobin, triglycerides, cholesterol and oxidative stress (catalase, SOD, Glutathione peroxidase) and cause delay of progression of type 2 diabetes. Diabetes can be prevented but very hard to be cured. Therefore probiotic fermented products can be considered as an important defense weapon to fight against type 2 diabetes in our daily life.

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