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EFFECTS OF PROBIOTIC SUPPLEMENTED YOGURT ON NEONATAL STREPTOZOTOCIN-INDUCED TYPE-2 DIABETIC RATS

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Abstract

Probiotics can be considered as biological agents that modify the intestinal microbiota as well as show several health benefits. The purpose of this study was to explore the effects of probiotics in neonatal STZ-induced type-2 diabetic rats. Following the biochemical analysis of probiotic bacteria, yogurt was prepared using cow milk and used to feed the experimental rats. Probiotic yogurt was supplemented to three rat groups, each with six rats, and the doses were 2 g in PYT1, 4 g in PYT2 and 6 g in PYT3 for 21 days. Fasting serum glucose, serum insulin, lipid profiles and liver glycogen levels were measured to investigate the probiotic effects on type 2 diabetic rats. It was observed that fasting serum glucose levels were significantly lower in case of PYT3 group ($p<0.05$) whereas a significant ($p<0.05$) upsurge of serum insulin levels was detected in PYT2 rat group. A significant ($p<0.05$) decrease in LDL level in PYT2 group and significantly ($p<0.05$) increased level of HDL was detected in PYT1 and PYT2 rat groups. However, there were no significant differences regarding triglyceride as well as total cholesterol levels among three probiotic yogurt supplemented rat groups. Hepatic glycogen content was 34.5%, 30.9% and 39.1% among the probiotic yogurt feed groups viz. PYT1, PYT2 and PYT3, respectively. Findings of this research suggest that application of probiotic yogurt can help to manage type-2 diabetes. Further study on clinical trial would be worthy to investigate for probiotic-based product improvement for treatment of type-2 diabetic patients.

Keywords: Diabetes, rats, probiotics, yogurt, metabolites

Introduction

Diabetes is a life-threatening chronic carbohydrate metabolism disorder in which blood glucose levels abnormally increase in the bloodstream due to a lack of insulin production (Sunday et al., 2022). International Diabetes Federation (IDF) stated that diabetes was anticipated to impact 463 million people in 2019 and the number is presumed to climb up to 578 million by 2030 and 700 million by 2045 (IDF, 2019). Type-2 diabetes mellitus (T2DM) is the most typical kind of diabetes, reckoning for 90% of all cases (Ortiz-Martínez et al., 2022; Cho et al., 2018). Retinopathy, nephropathy, and neuropathy are just a few of the issues associated with diabetes. Obesity, cataracts, erectile dysfunction, nonalcoholic fatty liver disease (NAFLD) are all risks associated with diabetes (Stenvers et al., 2019).

Probiotics are live microbial food additives that ensure health benefits surpassing rudimentary aliments when partaken in adequate proportions (Morelli et al., 2012). Probiotics have been studied for their potential to improve immunological function, reduce blood pressure, and improve lipids (Markowiak et al., 2017). Lactic acid bacteria (LAB) strains including *Lactobacillus* along with *Bifidobacterium* are known to be vital probiotic dosages. Fermented dairy products, namely yogurt with enough probiotic LAB, have been shown to have a number of benefits to health (Reid et al., 2005). Nowadays consumption of functional foods has been increased and functional food is one of the best strategies to overcome diabetes (Riezzo et al., 2005). Dairy foods have established health benefits. Dahi is a Bangladeshi homemade variant of yogurt as well as a potential source of probiotic bacteria and

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because of its numerous health benefits, it is regarded as a functional food i.e., antidiabetic (Yadav et al., 2007), antidiarrheal (Barai et al., 2018), anticarcinogenic (Brady et al., 2000), cholesterol-lowering (Ataie-Jafari et al., 2009), anti-allergic (Al Azad et al., 2020), antiatherogenic properties (Chawla et al.,1984; Abbas et al.,1992).

At present a significant number of people have type 2 diabetes as well as most of these individuals are living below the poverty line. They could not meet up their dietary requirements per day and a significant population remained malnourished. Because probiotic bacteria may have medicinal or preventive benefits, several probiotic products have been developed, including fermented milk drinks, yogurt, cheese, ice creams, sausages, probiotic juice and drinking water etc. with defined culture. This study aimed to analyze the effects of yogurt augmented with natural probiotic bacteria on neonatal Streptozotocin (STZ) induced diabetic rats.

Materials and methods

Probiotic bacterial identification

Ten probiotic bacteria were isolated from regional yogurt samples of Bangladesh and biochemical assay was performed based on the morphological (size, shape, and motility) and biochemical (Gram staining and catalase test) as well as physiological (pH tolerance, bile salt tolerance, NaCl tolerance, phenol tolerance, antibiotic activities) characteristics according to Hoque et al., 2010 and Barai et al., 2018.

Yogurt preparation

Milk was obtained from a residential cow farm then boiled above 100°C for 15 minutes before cooling to 40°C. A liquid culture of isolated probiotic bacteria was used to inoculate the bacteria at a concentration of 5% (v/v). The inoculated milk was then transferred into containers and incubated at 40°C for 12 hours in anaerobic condition. Raw coagulated yogurt was stored at 4°C in order to carry out onward applications (Barai et al., 2018).

Experimental Rats

In the present experiment, 36 mature Long Evans rats were used. The experimental rats were reared in the animal shed of the Bangladesh University of Health Sciences (BUHS), Dhaka, Bangladesh. Rats were kept at a constant room temperature of 22±50°C with a humidity of 40–70% at a natural 12-hour day-night cycle. Standard laboratory rat pellets were provided to the rats (as per formula and specifications of icddr,b) purchased from Jamuna Traders, Dhaka and ad libitum drinking water was provided. Rats were separated into six groups, each with six rats. The groups were normal water control (NWC), diabetic water control (DWC), Gliclazide treated diabetic rat (GT), 2 gm probiotic yogurt supplemented diabetic rats (PYT1), 4 gm probiotic yogurt supplemented diabetic rats (PYT2) and 6 gm probiotic yogurt supplemented diabetic rats (PYT3). Gliclazide was delivered orally to type-2 diabetic rats and the dosage was 20 mg/kg body weight. This study obtained informed consent from the ethical committee of Bangladesh University of Health Sciences (BUHS).

Preparation of type-2 diabetic model rats

A single intraperitoneal injection of Streptozotocin (STZ) in citrate buffer (10 ml) at a concentration of 90 mg/kg body weight was used for producing type-2 diabetes in rat pups (48 h old, average weight 7 g) according to Hassan et al., 2018; Hasan et al.,2019. The rat pups were administered an intraperitoneal injection of STZ solution with a dosage of 10 µl /g body weight. The three-month-old rats were first tested using a routine oral glucose tolerance test according to Barik et al., 2008. The experimental rats were considered as type-2 diabetic based due to their fasting blood glucose level > 7.0 mmol/l.

Biochemical analysis

To collect blood samples from rats that had been fasting, tail tip amputation was performed under diethyl ether anesthesia on "day 0". The tail was soaked using mild water (40°C) for 20 to 30 seconds for vasodilation shortly before amputation. To evade hemolysis, 0.2 ml of blood was accumulated with caution from the tail tip and placed in eppendorf tubes. Rat blood was obtained via heart puncture on the 21st day, after they had been decapitated. Obtained serum were divided into new eppendorf tubes for biochemical analysis. After centrifuging the blood samples at 3500 rpm for 15 minutes, 100 µl serum was frozen at -20°C until fasting serum insulin analysis. The glucose oxidase (GOD-PAP) technique was applied for determining serum glucose levels according to Trinder et al., 1969, which produces gluconic acid and hydrogen peroxide by oxidizing glucose. When peroxidase (POD) is

present, H_2O_2 produces a red violet hue when combined with a chromogenic oxygen acceptor, phenol aminophenazone. The glucose concentration was proportional to the sample's color intensity. The level of serum insulin was calculated via enzyme-linked immunosorbent assay (ELISA) following the protocol of the manufacturer (Crystal Chem. Inc. Downers Grove, IL, USA). The rats' serum cholesterol levels were estimated after enzymatic hydrolysis and oxidation. In the presence of phenol and peroxidase, the indicator quinoneimine was synthesized from hydrogen peroxide and 4 aminoantipyrine (Kendall et al., 1952). Calculation of low-density lipoprotein (LDL) was performed as stated by Friedewald et al., 1972. Serum HDL was assessed using the procedure stated by Kendall et al., 1952. By employing glycerol-3-phosphate oxidase, the serum triglyceride level was calculated using the GPO-PAP technique according to Fossati et al., 1982. The anthrone-sulfuric acid procedure was used to calculate liver glycogen levels, as per Vries et al., 1954.

Statistical Analysis

Statistical program SPSS was used to analyze the data (Windows Version 16, SPSS Inc., Chicago, Illinois, USA). The mean ± standard deviation was applied to represent the data. The paired t-test and one-way ANOVA were employed for the purpose of comparing the groups. The significance threshold was adjusted at $p<0.05$.

Results

Isolation of probiotic bacteria

Table 1 demonstrates that isolated bacteria were rod-shaped, gram-positive, catalase-negative, non-motile, and coagulase-positive. They exhibited resistance to low pH (3.0), NaCl (2-8%), phenol (0.1-0.4%), and bile salt (0.1-0.4%) concentrations (0.3%). The bacteria were identified as Lactobacillus spp. by all the tests performed.

Table 1. Physiological and biochemical features of the isolated bacteria

Isolate no.	Shape	Gram staining & Coagulase	Catalase & Motility	pH tolerance	Bile tolerance	NaCl tolerance				Phenol tolerance			
				pH 3.0	0.3%	2%	4%	6%	8%	0.1%	0.2%	0.3%	0.4%
1	rod	+	−	++	++	++	++	+	−	++	++	++	++
2	rod	+	−	++	++	++	++	+	−	++	++	++	++
3	rod	+	−	++	++	++	++	+	−	++	++	++	++
4	rod	+	−	++	++	++	++	+	−	++	++	++	++
5	rod	+	−	++	++	++	++	+	−	++	++	++	++
6	rod	+	−	++	++	++	++	+	−	++	++	++	++
7	rod	+	−	++	++	++	++	+	−	++	++	++	++
8	rod	+	−	++	++	++	++	+	−	++	++	++	++
9	rod	+	−	++	++	++	++	+	−	++	++	++	++
10	rod	+	−	++	++	++	++	+	−	++	++	++	++

++, the most excellent tolerant against the condition; +, the excellent tolerant against the condition; −, no tolerance against the condition.

Fasting serum glucose level in different groups of type-2 diabetic model rats

Measurement of fasting serum glucose levels is necessary to observe the glucose metabolism status**.** The effects of probiotic yogurt on glucose metabolism were evaluated by measuring the fasting serum glucose levels of different experimental groups. Probiotic yogurt treated group 3 (PYT3) indicated a significant ($p<0.05$) decrease in fasting glucose levels in contrast to the other probiotic treated groups (PYT1 and PYT2; Table 2**)**. On the contrary, the standard drug Gliclazide, which served as positive control (PC), showed 4.6% hypoglycemic effect. The results showed that PYT1, PYT2 and PYT3 decreased fasting blood glucose level by 14.6%, 24.8% and 23.1%, respectively at day 21 when compared with 'day 0' in type 2 diabetic rats. The results of serum glucose level also compared between probiotic supplemented yogurt treated groups with type 2 diabetic positive rat groups. However, no significant effects were obtained.

Effect of Probiotic Yogurt on serum insulin level

The serum insulin levels of experimental groups were examined to analyze the effect of probiotic yogurt on insulin levels. During the 21-day study period, PYT2 group showed a significant (p<0.05) increase in serum insulin levels in type 2 diabetic rats compared to PYT1 and PYT3 groups (Table 2). In Gliclazide treated group (positive control group), serum insulin level was amplified by 22.97% compared to 7.07% in the probiotic yogurt group, PYT2. However, interestingly, in PYT1 and PYT3 groups, serum insulin level was decreased by 7.72% and 28.07%, respectively at day 21 when compared with 'day 0'. The results of serum insulin levels were also compared between probiotic supplemented yogurt treated groups with type 2 diabetic positive rat groups. However, no significant effects were obtained.

Effects of Probiotic Yogurt on the Liver Glycogen

Controlling glucose metabolism primarily depends on the liver as it absorbs excess blood glucose as glycogen and/or regulates new glucose production via gluconeogenesis (Yoon et al., 2001). The present experiment investigated the impact of probiotic yogurt on hepatic glycogen level of type 2 diabetes model rats. All rats from separate test groups were executed after 21 days and their hepatic glycogen content was assessed. Table 2 depicts that there was 34.5%, 30.9% and 39.1% hepatic glycogen content in probiotic yogurt fed groups (PYT1, PYT2 and PYT3), respectively after 21 days of oral yogurt administration. The results of liver glycogen were also compared between probiotic supplemented yogurt treated groups with type 2 diabetic positive rat groups. However, no significant effects were obtained.

Table 2. Serum glucose, serum insulin and glycogen level of different experimental rat groups

Rat Groups	Serum glucose level (mmol/L)		Serum Insulin level (µg/L)		Glycogen level (mg/g)
	0 Day	21th Day	0 Day	21th Day	21th Day
NWC	6.12 ± 0.61 (100%)	6.26± 0.47 (102.2%)	-------	------	14.2±1.8
DWC	7.17 ± 1.13 (100%)	7.73 ± 1.14 (107.8%)	0.22±0.026 (100%)	0.213±0.023 (96.8%)	17.1±4.7 (100%)
GT	7.71 ± 0.75 (100%)	7.36 ± 1.34 (95.4%)	0.209±0.000 (100%)	0.257±0.062 (122.97%)	17.3±3.7 (101.1%)
PYT1	7.31 ± 0.17 (100%)	6.24 ±1.66 (85.4%)	0.259±0.008 (100%)	0.239±0.039 (92.28%)	23.0±1.0 (134.5%)
PYT2	7.64 ± 1.18 (100%)	5.75 ± 1.44 (75.2%)	0.198±0.003 (100%)	0.212±0.003* (107.07%)	22.4±1.1 (130.9%)
PYT3	8.93 ± 0.18 (100%)	6.87 ±1.19* (76.9%)	0.310±0.151 (100%)	0.223±0.03 (71.93%)	23.8±2.3 (139.1%)

*The outcomes are stated as Mean ± SD. Statistical analysis within groups was done using paired t-test. SD, Standard Deviation; NWC: Normal Water Control; DWC: Diabetic Water Control; GT: Gliclazide Treated Diabetic Rat; PYT1:2 gm probiotic yogurt supplemented diabetic rat group; PYT2:4 gm probiotic yogurt supplemented diabetic rat; PYT3:6 gm probiotic yogurt supplemented diabetic rat group. * indicates statistically significant (p < 0.05).*

Plasma lipid profiles of type-2 diabetic model rats

The level of LDL cholesterol was reduced (p<0.05) in PYT2 group and significant (p<0.05) upsurge in HDL cholesterol level in PYT1 as well as PYT2 groups were observed following a 21-day period of constant feeding (Table 3). However, there were no noteworthy differences in triglyceride and total cholesterol levels among the three probiotic yogurt supplemented groups. The results of lipid profiles were also compared between probiotic supplemented yogurt treated groups with type 2 diabetic positive rat groups. However, no significant effects were observed.

Table 3. Plasma lipid profiles of different experimental rat groups

Rat Groups	TG (mg/dL)		Cholesterol (mg/dL)		HDL (mg/dL)		LDL (mg/dL)	
	0 Day	21th Day	0 Day	21th Day	0 Day	21th Day	0 Day	21th Day
NWC	87.2±16.9 (100%)	56.6±12.8 (64.9%)	51.0±2.5 (100%)	38.4±4.9 (75.2%)	16.4±4.6 (100%)	17.9±5.4 (109.1%)	17.1±6.2 (100%)	16.2±3.2 (94.7%)
DWC	51.1±12.6 (100%)	82.9±15.9 (162.2%)	50.7±10.1 (100%)	52.7±10.1 (103.9%)	21.4±6.1 (100%)	21.3±4.1 (99.5%)	21.0±12.0 (100%)	17.9±6.1 (85.2%)
GT	68.3±29.4 (100%)	93.6±18.6 (137.0%)	50.9±5.5 (100%)	58.3±10.5 (114.5%)	20.1±4.5 (100%)	20.9±3.1 (103.9%)	20.1±6.9 (100%)	20.2±7.6 (100.4%)
PYT1	99.8±45.3 (100%)	74.2±21.5 (74.3%)	51.1±4.9 (100%)	62.8±6.2 (122.8%)	20.0±4.0 (100%)	25.7±3.2* (128.5%)	17.5±5.1 (100%)	24.6±7.5 (140.5%)
PYT2	94.2±33.6 (100%)	79.6±11.2 (84.5%)	54.4±3.0 (100%)	57.9±7.3 (106.4%)	23.0±4.2 (100%)	28.0±5.6* (121.7%)	18.7±3.2 (100%)	15.6±3.1* (83.4%)
PYT3	105.4±29.9 (100%)	89.3±22.3 (84.7%)	50.2±6.0 (100%)	60.1±7.6 (119.7%)	24.5±4.7 (100%)	22.5±5.9 (91.8%)	10.1±4.3 (100%)	17.5±2.6 (173.2%)

*The outcomes are stated as Mean ± SD. Statistical analysis within groups was done using paired t-test. SD, Standard Deviation; NWC: Normal Water Control; DWC: Diabetic Water Control; GT: Gliclazide Treated Diabetic Rat; PYT1: 2 gm probiotic yogurt supplemented diabetic rat group; PYT2: 4 gm probiotic yogurt supplemented diabetic rat; PYT3: 6 gm probiotic yogurt supplemented diabetic rat group. * indicates statistically significant ($p < 0.05$).*

Body weights of different rat groups

The findings of this study reveal that after 21 days of feeding, body weight of the rats did not differ significantly. All the rat groups had a proclivity status for gaining body weight. The variations in the body weight of rats have shown in Table 4. The results of body weights were also compared between probiotic supplemented yogurt treated groups with type 2 diabetic positive rat groups. However, no significant effects were recorded.

Table 4. Body weight of different experimental rat groups

Rat groups	Body weight (gm)			
	Day 0'	Day 7'	'Day 14'	"Day 21'
NWC	238 ± 10 100%	270 ± 8	283±10	304±13 127%
DWC	259±42 100%	252±46	257±42	264±42 101%
GT	254±30 100%	251±32	255±29	257±27 101%
PYT1	208±19 100%	207±20	214±20	206±20 99%
PYT2	205±35 100%	203±32	211±34	206±27 100.4%
PYT3	208±16 100%	215±14	218±13	210±14 100.9%

*The outcomes are stated as Mean ± SD. Statistical analysis within groups was done using paired t-test. SD, Standard Deviation; NWC: Normal Water Control; DWC: Diabetic Water Control; GT: Gliclazide Treated Diabetic Rat; PYT1: 2 gm probiotic yogurt supplemented diabetic rat group; PYT2: 4 gm probiotic yogurt supplemented diabetic rat; PYT3: 6 gm probiotic yogurt supplemented diabetic rat group. * indicates statistically significant ($p < 0.05$).*

Discussion

Insulin insufficiency are the consequences of pancreatic β-cell failure characterized Type 2 diabetes (IDF, 2019). Several researches revealed that, between diabetic and non-diabetic individuals, gut microbiota was altered in diabetic patients and gut which did contribute to the onset of diabetes as well as metabolic disorders (Larsen et al., 2010, He et al., 2015). Antimicrobial agents or metabolic chemicals produced by probiotics prevent the development of additional microbes (Spinler et al., 2008; O'Shea et al., 2012). On the intestinal mucosa, they compete with other gut microbes for receptors and binding sites, as well as change the gut environment (Collado et al., 2007).

Probiotic bacteria were isolated and characterized by biochemical screening process in the present study. They were cultured on the de Man, Rogosa and Sharpe (MRS) agar media. These isolates were sub-cultured after growing on MRS agar medium, and colonies were chosen for physiological and biochemical analysis. After that for the purpose of determining probiotic characteristics, ten isolates were chosen. The most crucial characteristic of probiotic bacteria is the ability to resist low pH like gastric juice (pH 1.5-3) as they move through the stomach (Chou et al., 1999). All of the isolates showed resistant to low pH in the present study. Bile tolerance is among the most essential characteristics of probiotic bacteria (Walker et al., 2000). Bile salt tolerance activity of probiotic isolates showed more or less nearly equal resistance against 0.05%, 0.1%, 0.15% and 0.3% artificial bile acid concentration after 0, 2, 4 and 24 hours of incubation, respectively (Prasad et al., 1998; Hossain et al., 2018). Findings of current study shows that all of the isolates were able to withstand at 0.3% bile salt concentration. According to Hoque et al., 2010 and Barai et al., 2018, yogurt was used to isolate *Lactobacillus* spp. and was analyzed at various NaCl concentrations (1-10%) and were tolerant to 1-9% NaCl. The present study showed that all the isolated bacteria were able to survive at 2% and 4% NaCl, but less survival ability was observed at 6% and 8% NaCl concentrations. It is reported that, bacteriostatic action in some microorganisms occurred at 0.4% concentration of phenol (Xanthopoulos et al., 2000). In findings of present study, all the isolated bacteria were able to survive at 0.1%, 0.2%, 0.3% and 0.4% phenol concentrations, respectively. It has been reported that, probiotic bacteria ferment sugars (glucose, sucrose, maltose, lactose) except sorbitol and mannitol which corresponds to the findings of present study (Ghanbari et al., 2009).

Appropriate experimental models are necessary to test the effect of various bio-therapeutic agents. Type-2 diabetes animal models were obtained either spontaneously, surgically or using chemicals or a combination of these techniques. It has been reported that neonatal streptozotocin-induced Long Evans rats showed the mimic effects on human diabetes with fair parallelism. However, these effects are manifestations of insulin secretory dysfunction rather than insulin resistance (Asrafuzzamanet al., 2018; Mudi et al., 2017) and for this reasons neonatal STZ-induced diabetic rats were chosen in the present study.

Determination of treatment doses of probiotic yogurt is so much important. In the present study, the treatment doses of probiotic yogurt were determined as reported by the previous studies (Delia et al., 2012). Yadav et al., 2008 reported that, in STZ-induced type 2 diabetic rats, oral feeding of probiotic dahi (15 g/day/rat) delayed insulin secretion, reduced total cholesterol, triglycerides, LDL and VLDL cholesterol, however, enhanced HDL cholesterol levels. An equivalent pattern was demonstrated in the present research where probiotic high doses yogurt fed treatment group (PYT3) decreased fasting serum glucose level significantly ($p<0.05$). Administration of *L. plantarum* for 20 weeks at 25×10^8 CFU/day lowered blood glucose level in a high fat diet female C57BL/6 J mouse (Andersson et al., 2010). Lu et al. (2010) reported that administration of *L. reuteri* for 4 weeks at 1×10^9 CFU/day lowered blood glucose level and glycated hemoglobin in a male Sprague–Dawley diabetic rats. In this study, a significant ($p<0.05$) decrease in the LDL cholesterol level along with an increased ($p<0.05$) HDL cholesterol level was observed. It was found that, treating with diet supplemented with 15% of dahi for 8 weeks, lowered blood glucose level in male Wistar rats fed a high fructose diet (Yadav et al., 2007). Administration of *Bifidobacterium adolescentis* for 12 weeks increased insulin sensitivity and decreased body weight in male Wistar rats when provided a high fat diet (Chen et al., 2012). The present study revealed a significant ($p<0.05$) upsurge in serum insulin levels in case of probiotic supplemented treatment groups. Body weight gain was slowed or ceased 8 weeks later in case of *Lactobacillus gasseri* BNR17 administration on a type 2 diabetes mouse model (Yun et al., 2009). In the findings of the present study, all the probiotic yogurt supplemented rat groups showed similar trend of body weight after 2 weeks compared to other experimental rat groups. Human clinical trials of probiotic capsules or yogurt were conducted and yielded mixed outcomes. Six weeks of probiotic yogurt consumption up surged blood glucose levels dramatically (Ruan et al., 2015).

In the present research, antidiabetic impact of probiotics was determined on streptozotocin induced type-2 rats. It was a laboratory animal study and the duration of the experiment was 3 weeks. From this experiment, a significant ($p<0.05$) decrease in fasting serum glucose levels was observed in case of PYT3 group and a significant ($p<0.05$) increase in serum insulin levels in PYT2 group. Due to some unknown factors it was observed that, blood glucose levels decreased in PYT3 group, however, the corresponding insulin level was also decreased. GT used in the present experiment might not have had the desired effects for which the GT group did not show the expected result. It may me mentioned that, the experimental groups were treated with high doses of yogurt, which might be resulted increased levels of liver glycogen. It was observed that probiotic enriched yogurt lowered the blood glucose

level and increased the insulin, HDL and glycogen level. Changes in HDL and LDL levels are not consistent between the experimental groups. However, unknown factors might be responsible for this particular results. In addition, no significant results were obtained regarding increased body weight among the experimental groups which is corroborated by the findings of other authors.

Further study regarding molecular characterization and proper gene identification would be worthy to investigate. Moreover, probiotic-based food may be developed as a bio-therapeutic agent for type 2 diabetic patients.

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Conflict of Interests

The author declares no conflict of interest.

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