

Original Article

Effect of *Lactobacillus delbrueckii* on Blood Sugar in Diabetic MiceReza Dolatyari Eslami¹ M.Sc., Asghar Tanomad^{2*} Ph.D.
Jamal Hallajzadeh² Ph.D., Mehdi Ariana³ M.Sc.¹Department of Microbiology, Higher Education Institute of Rabe Rashid, Tabriz, Iran.²Department of Microbiology, Maragheh University of Medical Sciences, Maragheh, Iran.³Department of Microbiology, University of Tehran, Tehran, Iran.

A B S T R A C T

Article history

Received 8 Apr 2016

Accepted 3 Sep 2016

Available online 29 Oct 2016

Key words

Diabetic mice

Lactobacillus delbrueckii

Probiotic

Background and Aims: Probiotics are defined as live microorganisms that are beneficial to human health. Probiotics are commonly found in some foods such as dairy products or complementary tablets. A recent research shows that probiotics have a significant role in diabetes type 1 and 2 treatment. Among the variety of lactic acid bacteria, *Lactobacillus* strains are well known in probiotics for their different properties. The aim of this study is to investigate the effect of *Lactobacillus delbrueckii* PTCC1057 on diabetic mice blood sugar.

Materials and Methods: sample mice were selected from the age range of 6 to 8 weeks. Then for converting the normal mouse to diabetic type, 180 mg/kg streptozotocin was injected intraperitoneally. 10 days later, the blood sugar of the mice was measured and the mice with higher than 200 mg/dl of blood sugar were considered as diabetic mice. Finally, the diabetic mice were divided into two groups of experimental and control for next investigation. For a survey of probiotics effect on blood sugar, an amount of 3×10^6 CFU of *L. delbrueckii* was fed by gavage feeding approach daily during 4 weeks and the blood sugar level was determined at the end of the week after streptozotocin injection.

Results: the result showed that the diabetic mice blood sugar level significantly decreased conspicuously by feeding *L. delbrueckii* in comparison to diabetic control group.

Conclusion: The result of this study suggests that *L. delbrueckii* can be impressive in diabetes treatment. However, another clinical study is needed for proving the exact effect of this probiotic on diabetes.

Introduction

Diabetes is one of the common metabolic disorders all around the world. According to World Health Organization report on diabetes, almost 171 million people are contracted to diabetes and this number will increase till year 2030 [1]. Diabetes is a metabolic disorder that is divided into two separate types of 1 and 2. Betapancreas cells are responsible for insulin production in human body. Insulin can attach to specific ligands on the cell surface and increase glucose absorption by cell and decrease blood glucose. Therefore, reducing the insulin level in blood caused by beta pancreas dysfunction can lead to glucose accumulation in blood and hyperglycemia disease [2]. Hyperglycemia itself is cause of some side effects such as microvesicular diabetes and capillary devastation that harm organs like kidney, heart and eyes [3]. Nowadays different methods are applied for treatment that concomitant with some side effects.

Probiotics are viable microorganisms that have a healthy effect on a human if they are used properly [4]. Among the variety of probiotics, *lactobacillus* strains are used routinely in food products. Some regulations such as stimulation of immune system, inhibition of pathogen attachment, reduction of the toxic effect of pathogenic microorganisms and stimulating of the small intestine are some consequences of lactobacillus strains in the body [5-7].

The lactic acid bacteria can have potential application in bio-medical treatment. Some of the uses are in managing lactose intolerance,

prevention of colon cancer, lowering cholesterol, lowering blood pressure, improving immune function and preventing infections, antibiotic-associated diarrhea, reducing inflammation, irritable bowel syndrome and colitis, managing urine-genital health. Also it reduces risk of certain cancers, detoxify carcinogens, suppress tumors, lower serum cholesterol concentrations, reduce blood pressure in hypertensive, synthesize nutrients, increase nutrient bioavailability, improve urine-genital health, optimize effects of vaccines, treat milk allergies, improve mineral absorption. Lactic acid bacteria have a high potential as probiotics [8, 9].

According to the probiotics effects on human health, investigation of blood sugar lowering effect of *Lactobacillus delbrueckii* (*L. delbrueckii*) as a common probiotic in foods can be impressive. The objective of this study is a survey of blood sugar lowering effect of *L. delbrueckii* on the diabetic mouse with intraperitoneally injection.

Materials and Methods

Bacterial strains and medium cultures

L. delbrueckii PTCC1057 was used for investigation of blood sugar lowering effect of probiotic. The *L. delbrueckii* PTCC1057 was purchased from Iranian Research Organization for Science and Technology. For preparation of bacterial suspension, *L. delbrueckii* PTCC1057 was cultured in MRS medium and placed in 37°C and incubated for 18-24 hours. Further investigation was performed on strain

by catalase and oxidase tests. Then in order to make desired suspension of *L. delbrueckii* PTCC1057, the strain was cultured in MRS broth and incubated in 37°C for 18-24 hours. After 24 hours, the samples were centrifuged at 2500 rpm and 4°C for 10 minutes for biomass separation. Finally, biomass was washed by physiological serum 3 times and prepared as suspension.

Then McFarland 0.5 standards were used as a reference to adjust the turbidity of bacteria. This adjustment is performed by spectrophotometer at 625 nm that showed absorption of 0.08-0.13 that is equal to 1.5×10^8 CFU/ml of bacteria. Finally, the bacterial suspension was mixed with some carrot juice for feeding the mouse.

In Vivo analysis of blood sugar lowering effect of *L. delbrueckii*

For the invivoanalysis of mouse, the number of 15 mice is purchased from Pastor Institute of Iran with the weight of 20-25 gr and age range of 6-8 weeks and is kept in a normal condition of 12 hours light and 12 hours dark. After maintaining the mouse with standard food and water and conformity with the laboratory condition, they were randomly divided into 3 groups of 5 members.

Streptozotocin (Sigma) was injected intraperitoneally in a dose of 180 mg per kg body weight into two separately groups. Ten days after injection, the amount of blood glucose is measured by glucometer and commercial Kit. The blood samples are obtained through the mouse tail. The mouse with the blood glucose of more than 200 mg/dl is considered as diabetic mouse (the

blood glucose level of non-diabetic mouse is considered lower than 120 mg/dl).

1. First group (Non-diabetic control): 100 micro-liters of carrot juice lacking *L. delbrueckii* gavaged each mouse every day for 4 weeks.

2. Second group (Diabetic control): 100 micro-liters of carrot juice lacking *L. delbrueckii* gavaged each mouse every day for 4 weeks.

3. Third group (Diabetic experimental): 100 micro-liters of carrot juice containing 3×10^7 CFU/ml of *L. delbrueckii* gavaged each mouse every day for 4 weeks.

The protocol was approved by Ethics Committee of Maragheh University of Medical Sciences, Maragheh, Iran.

Statistical Analysis

The results were analyzed by Friedman test in SPSS software version 21.

Results

The result of blood sugar measurement at the end of each week indicates that the blood sugar of experimental group conspicuously decreased in comparison to control group. According to the results, after 4 weeks gavage of *L. delbrueckii* not only the blood sugar level decreased but also the sign of illnesses such as polydipsia and diarrhea were obviated. As well, the amount of reducing blood sugar in mouse that have been gavaged with *L. delbrueckii* was equal to 100-200 unit whereas, in the diabetic group, blood sugar was increased gradually (Table 1).

Table 1. Blood sugar results (Mg/dl) after 1, 2, 3, 4 and 5 weeks gavaging in experimental and control groups mice sera

Groups	Blood sugar after 1 week	Blood sugar after 2 weeks	Blood sugar after 3 weeks	Blood sugar after 4 weeks	Blood sugar after 5 weeks
First group					
Non-diabetic (control)					
1	120	118	118	108	116
2	121	110	115	100	106
3	118	116	110	114	116
4	108	101	98	110	103
5	110	107	102	111	108
Second group					
Diabetic (control)					
1	205	234	289	315	388
2	286	368	458	479	617
3	230	348	422	458	583
4	268	291	296	387	556
5	225	242	278	296	446
Third group					
Diabetic (experimental)					
1	297	207	187	224	202
2	287	180	176	158	136
3	556	458	406	316	264
4	196	118	118	126	164
5	268	221	176	158	156

The results showed that blood sugar significantly decreased in diabetic experimental group compared to diabetic control group ($p < 0.5$) (Table 2).

Table 2. Mean of blood sugar (Mg/dl) after 1, 2, 3, 4 and 5 weeks gavaging in three groups

Variable	Time (Weeks)	Mean ± SD	P-Value
Diabetic control	1	242.80±33.21	<0.0001
	2	296.60±60.56	
	3	348.60±84.65	
	4	387.00±82.11	
	5	518.00±96.15	
Diabetic experimental	1	320.80±137.29	0.014
	2	236.80±129.81	
	3	212.60±111.45	
	4	196.40±75.78	
	5	184.40±50.53	
Non-diabetic control	1	115.40±5.98	0.124
	2	110.40±6.88	
	3	108.60±8.47	
	4	108.60±5.27	
	5	109.80±5.93	

Discussion

Matsuzaki and colleagues in their study in 1997 on KK-A^y mice showed that by gavaging *L. casei* for 16 weeks, 5 times and each time 2 mg the plasma glucose levels in the mice were reduced. [10]. In another study in 2003, Tabuchi and his colleagues found that blood sugar level in diabetic rats, with streptozotocin injection, reduced by eating *Lactobacillus GG* [11]. In 2007, Yadav and his colleagues studied the anti-diabetic effect of Dahi, a probiotic product containing *Lactobacillus casei* and *L. acidophilus*, on diabetic rats with fructose (type 2 diabetes). It was found that Dahi probiotics reduced blood glucose level and glucose intolerance [12]. Yadav and colleagues in 2007 in another study also showed that the consumption of Dahi probiotic can even decrease blood sugar levels in diabetic rats with streptozotocin (type 1 diabetes) by strengthening the antioxidant system and thereby reducing cell damage attributed beta pancreas [13]. In 2007, Laleye et al. found that nano-products containing *Lactobacillus* reduce blood glucose level in the diabetic rats with alloxan [14]. Harisa et al. in a study in 2009 showed that oral administration of *Lactobacillus acidophilus* by alloxan-induced diabetic rats, can reduce oxygen free radicals which leads to modulating nitric oxide in the animal body. Nitric oxide is an important intermediate in the secretion of hormones and stimulates the immune system. The oral administration of *Lactobacillus acidophilus* leads to lower plasma glucose level in alloxan-induced

diabetic rats [15]. A study in 2011 by Omid and his colleagues showed that blood sugar levels in diabetic rats with streptozotocin injection after 14 days of treatment with *Lactobacillus acidophilus* mixed in wines was reduced [16]. The randomized double-blind clinical study on 60 patients with type 2 diabetes, 2 experimental and control groups with 30 individuals in each were created. The experimental group received daily 300 gr of probiotic yogurt for 6 weeks, but the control group received daily 300 grams of non-probiotic for 6 weeks and a significant reduction in blood glucose level was observed in the experimental group [17]. In 2013, Jafari et al. conducted a study entitled as "Effect of *Lactobacillus casei* strain TD2 native to Iran on blood sugar level in diabetic male Wistar rats". In this study, the streptozotocin-induced diabetic rats were fed by gavaging *Lactobacillus casei* TD2, 10⁹ CFU, for 21 days. The result showed that *Lactobacillus casei* TD2 significantly reduced blood glucose levels in diabetic mice, while the healthy mice receiving probiotics did not show any changes in blood glucose level [18].

In this study the effect of oral administration of probiotic *Lactobacillus delbrueckii* subsp. *Lactis* PTCC1057 on blood glucose level of streptozotocin-induced diabetic mice was studied. The results showed that blood glucose levels in diabetic mice significantly reduced after 28 days of taking probiotics compared to the diabetic control group that did not receive probiotics. But blood sugar

level did not reach to normal levels of blood glucose in non-diabetic mice, which admitted that *Lactobacillus delbrueckii* PTCC1057 completely would not cure diabetes, but it can be used as adjuvant therapy. Researchers think that the reduction of blood sugar level in the diabetic patients by consumption of probiotics is due to the establishment of probiotics in intestines of the patients. These organisms increase the amount of glucose consumption in the gut, thus reducing the amount of glucose absorbed into the blood [11-14].

Conclusion

According to the results of this study, probiotics can help to treat diabetes. However, further research is required to ensure using

safty of these microbes. Because probiotics consumption may disrupt intestinal flora and have long-term harmful effects on the health of the host, but what is already clear is that oral administration of probiotics have a beneficial effect on reducing blood sugar levels and consequently reducing the severity of diabetes complications which can be used as adjuvant therapy for diabetic patients.

Conflict of Interest

Authors declare no conflict of interest.

Acknowledgement

There is no Acknowledgement to declare.

References

- [1]. Wild S. Global prevalence of diabetes estimates for the year 2000 and projections for 2030. *Diabetes Care*.2004;27(5): 1047-53.
- [2]. Farvid M. The impact of vitamin C and E, magnesium and zink on glycemic control and insulin resistance in type II diabetic patients. *Tehran University Medical Journal* 2006; 64(10):67-75.
- [3]. Caballero AE, Arora S, Saouaf R, Lim SC, Smakowski P, Park JY, et al. Microvascular and macrovascular reactivity is reduced in subjects at risk for type II diabetes. *Diabetes* 1999; 48: 1856-862.
- [4]. Lin WH. Different probiotic properties for *Lactobacillus fermentum* strains isolated from swine and poultry. *Anaerobe* 2007;13(3-4): 107-13.
- [5]. Farnworth ER, Mainville I, Desjardins MP, Gardner N, Fliss I, Champagne C. Growth of probiotic bacteria and bifidobacteria in a soy yogurt formulation. *International J Food micro*.2007; 116:174-81.
- [6]. Sheehan VM, Ross P, Fitzgerald GF. Assessing the acid tolerance and the technological robustness of probiotic culture for fortification in fruit juice. *IFSET*. 2007;8(2): 279-84.
- [7]. Isen J, Huang Y, Lin Y, King V. Freezing resistance improvement of *Lactobacillus reuteri* by using cell immobilization. *J microbial Methods* 2007;70(3): 561-64.
- [6]. Homayouni Rad A. Therapeutical effects of functional probiotic, prebiotic and symbiotic foods. 1st ed. Tabriz: Tabriz University of Medical Science,2008.
- [9]. Kaur IP, Kuhad A, Garg A, Chopra K. Probiotics: delineation of prophylactic and therapeutic benefits. *J Med Food* 2009;12(2): 219-35.
- [10]. Matsuzaki T. Ant diabetic effects of an oral administration of *Lactobacillus casei* in a non-insulin-dependent diabetes mellitus (NIDDM) model using KK-A^y mice. *Endocrine Journal* 1997; 44(3):357-65.
- [11]. Tabuchi M, Ozaki M, Tamura A, Yamada N, Ishida T, Hosoda M, et al. Ant diabetic effect of *Lactobacillus GG* in streptozotocin-induced diabetic rats. *JSBA*.2003; 67(6): 1421-424.
- [12]. Yadav H. Ant diabetic effect of probiotic dahi containing *Lactobacillus acidophilus* and *Lactobacillus casei* in high fructose fed rats. *Nutrition Journal* 2007; 23(1): 62-8.

- [13]. Yadav H. Oral administration of Dahi containing probiotic *Lactobacillus acidophilus* and *Lactobacillus casei* delayed the progression of streptozotocin-induced diabetes in rats. *Journal of Dairy Research* 2008;75(2):189-95.
- [14]. Laleye SA, Igbakin AP, Akinyanju JA. Ant diabetic effect of no-no (a Nigerian fermented milk) on alloxan-induced diabetic rats. *American Journal of Food Technology* 2008; 3(6): 394-98.
- [15]. Harisa G. Oral administration of *Lactobacillus acidophilus* restores nitric oxide level in diabetic rats. *Aust J Basic and Appl Sci.* 2009; 3(3): 2963-969.
- [16]. Omidi B, Amoozgar M, Fazeli M, Mortazavi P. Ant diabetic effect of probioticated Persian yellow carrot juice with *Lactobacillus acidophilus*. *Journal of comparative Pathology* 2011; 8(1):395-402.
- [17]. Ejtahed H, Mohtadi Nia J, Homayouni Rad A, Niafar M, Asghari Jafarabadi M, Mofidi V. The effects of probiotic and conventional yogurt on diabetes markers and insulin resistance in type 2 diabetic patients. *Iranian Journal of Endocrinology and Metabolism* 2011;13(1):1-8.
- [18]. Jafari P, Akbari N, Badkooyeh H. The effect of *Lactobacillus casei* strain TD 2 Iran on blood glucose in diabetic male wistar rats. *Iranian journal of animal physiology and development* 2013;6(3):17-22.