International Journal of Medical Laboratory 2016;3(3):198-204.

International Journal of Medical Laboratory
Shahid Sadoughi University of Medical Sciences

Original Article

Effect of *Lactobacillus delbrueckii* on Blood Sugar in Diabetic Mice

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Article history
Received 8 Apr 2016
Accepted 3 Sep 2016
Available online 29 Oct 2016

Key words
Diabetic mice
Lactobacillus delbrueckii
Probiotic

A B S T R A C T

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 3×106 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.

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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 37ºC and incubated for 18-24 hours. Further investigation was performed on strain

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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 37ºC for 18-24 hours. After 24 hours, the samples were centrifuged at 2500 rpm and 4ºC 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).

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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	
	s	108.60±8.47	
	4	108.60±5.27	
		109.80±5.93	

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Discussion

Matsuzaki and colleagues in their study in 1997 on KK-AY 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 Omidi 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*, 10^9 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

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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 diabeticpatients 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.

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