Journal of Endocrinology, Metabolism and Diabetes of South Africa 2017; 22(3):47–50
https://doi.org/10.1080/16089677.2017.1378460

JEMDSA
ISSN 1608-9677 EISSN 2220-1009

RESEARCH

Investigating the effects of *Lactobacillus casei* on some biochemical parameters in diabetic mice

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Aims: Diabetes mellitus is a metabolic disorder characterised by inadequate pancreatic insulin secretion or the insulin present being unable to perform its function properly. Consistent with the beneficial effects of probiotics and their ability to lower glucose levels, an impact on diabetes treatment is also expected. The aim of this study was to evaluate the effect of *Lactobacillus casei* on various either biochemical parameters in a diabetic mice model.
Methods: In the present study, 24 mice were divided into diabetic and control groups. Further, each group was categorised into two subgroups. The diabetic and control subgroups were fed carrot juice or *Lactobacillus casei* in carrot juice. Diabetes was induced by streptozotocin (STZ). For 30 days, the mice were fed 2 ml carrot juice, and *Lactobacillus casei* in carrot juice (with lactobacillus 10^9 cfu/ml) by gavage. Then, blood samples were collected to assay biochemical parameters.
Results: The results of this study showed that *Lactobacillus casei* (ATCC39392) significantly reduced blood glucose (BG) levels in diabetic mice receiving the probiotic, but did not cause a significant change in BG levels in control mice receiving the probiotic. When comparing insulin, insulin-like growth factor I (IGF-I) and C-peptide in the four groups, it was found that insulin and C-peptide were significantly different in all groups except for the control group treated with a mixture of probiotic *Lactobacillus casei* and carrot juice.
Conclusion: Our results showed that probiotic *Lactobacillus casei* effectively reduces BG levels in diabetic mice treated with this bacterium.

Keywords: diabetes, *Lactobacillus casei*, probiotic

Introduction

Diabetes mellitus (DM) is one of the most common metabolic diseases in the world.[1,2] According to a WHO report, nearly 171 million people are afflicted with this disease. It is predicted that by 2030 the figure will hit 366 million. Any disorder in the function of pancreatic β-cells will lead to hyperglycaemia whereby if the immune system interferes in the appearance of such disorders it is called diabetes type 1 and if receptive cells resist against insulin it is called diabetes type 2.[4,5] This disease may lead to long-term complications like nephropathy, neuropathy, retinopathy, brain stroke and heart attack and even malformation.[6–9] Nowadays, different approaches are employed to treat this disease.[10] Discovering new methods to prevent and treat DM has been highly prioritised. Meanwhile, probiotics that contain live microorganisms can have healing effects provided that the host uses a sufficient dose.[11] Bacteria producing lactic acid, especially lactobacillus, are commonly considered to be part of the digestive system ecosystem. Proven potential effects of probiotic microorganisms include aiding digestion of lactose in the intestine, alleviating diarrhoea and constipation, decreasing cholesterol level, controlling colon cancer, reinforcing of the immune system, stimulating intestinal microflora growth, preventing increases in allergic reactions, and restraining pathogen growth in the intestine.[3,12] Hence, in line with the positive effects of probiotics and increasing spread of DM and scarcity of studies concerning this issue, the present study probes the effects of *Lactobacillus casei* on biochemistry factors in diabetic Syrian mice so as to determine the effects of using probiotics to help treat DM.

Materials and methods

In this study, 24 female mice whose weight ranged from 28 to 32 grams were obtained from the research centre of Tabriz University of Medical Sciences. Prior to the experiment, in order to make the mice under study compatible, they were kept at 25 ± 2°C under equal conditions of light and dark in special cages where they had access to sufficient water and typical food. The mice were then categorised into two groups and a further two subgroups. The diabetic and control subgroups received carrot juice and *Lactobacillus casei* in carrot juice (Figure 1). The animal care methods and experimental procedures employed were approved by the Animal Ethics Committee of the Tabriz University of Medical Sciences.

In order to induce DM in the mice, streptozotocin (STZ) with the trade name Zanosar®, purchased from Sigma Aldrich (St Louis, MO, USA) and dissolved in a citrate buffer, was injected intraperitoneally at a dose of 60 mg/kg of body weight. Ten days after injection, some signs like overeating, over-drinking and frequent urination were observed. To ensure that the mice were diabetic, blood glocuse (BG) levels were measured with a glucometer and mice with a blood glucose level exceeding 200 mg/dl were considered diabetic.

Lactobacillus casei (ATCC39392) was obtained from collection and industrial bacteria of Iran regional centre as lyophilised ampoules. With a sterile Pasteur pipette, 0.3–0.4 ml sterile solution (growth medium for liquid or normal saline) was added to the dried material in the ampoule and was mixed to ultimately a uniform suspension. Then, the bacterium was cultivated in MRS

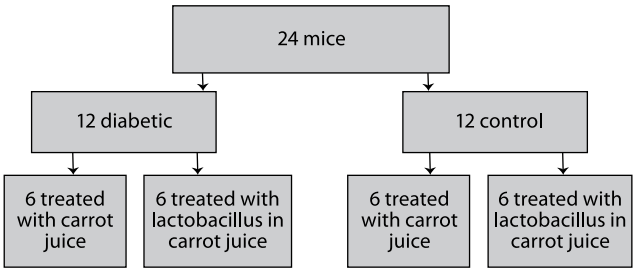


Figure 1: Categorisation of mice into two groups and two subgroups in this study.

cultivation broth. Finally, through gram staining and catalase and oxidase tests, it was ascertained that the mentioned bacterium was a gram-positive bacillus of lactobacillus. In order to gavage-feed the mice, 5 ml bacterium was cultivated in MRS cultivation broth. After 48 hours' incubation in an incubator containing 10% carbon dioxide, the bacteria were separated in a centrifuge at a speed of 6000 rpm for 15 minutes. After washing with normal saline and centrifuging again, the pipes containing sterile distilled water were filled with bacteria until an opacity equalling 0.5 using McFarland's standard was reached. Meanwhile, light absorption of the microbial suspension at a wavelength of 600 nanometres was determined and recorded. The pour plate method was used to determine the number of bacteria in microbial suspension and in fact to attain colony forming units/ml (CFU/ml). Ultimately, a bacterial suspension of 10^9 cfu/ml density was attained. Then, 1 ml of bacterial suspension was blended with 4 ml carrot juice (*Daucus carota*). Thereafter, 2 ml of this mixture was fed to the second healthy group and the fourth diabetic group in the form of gavage every day for 30 days. The healthy and the diabetic groups were fed carrot juice only for 30 days.[13]

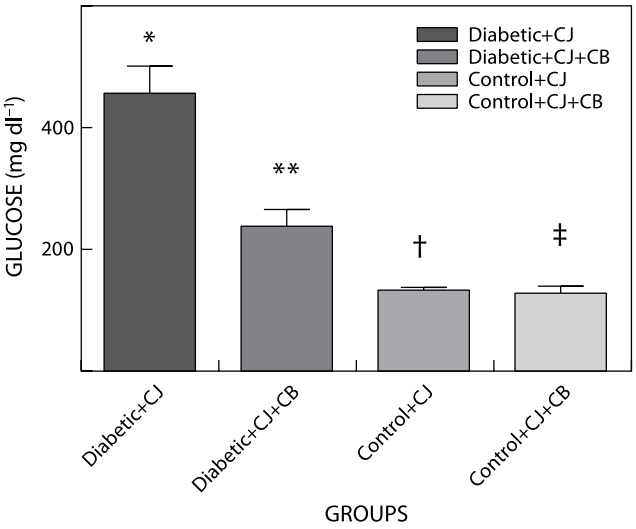
At the end of the treatment period, after 12 hours' fasting, mice were anaesthetised and a blood specimen collected (the mice were weighed before and after treatment).

The collected blood was centrifuged at 3000 rpm to separate blood serum. Measurement of blood glucose level was carried out by the Enzymatic-Colorimetric GOD-PAP method. Insulin, insulin-like growth factor I (IGF-I) and C-peptide were measured using the ELISA technique (kit from Monobind Inc, Lake Forest, CA, USA).

All of the statistical methods were done using SPSS16® software (SPSS Inc, Chicago, IL, USA). The graphs were drawn using GraphPad Prism® (version 6) software (San Diego, CA, USA); variables were expressed as mean ± SD.

Results

To compare data in various groups the one-way ANOVA test was used. The distribution of all data in this study was normal. In order to find out the various group changes, a Tukey post hoc test was performed to make a pairwise comparison. Results showed that all the groups had significant differences in comparison one another (p-value = 0.001), except that the healthy control group receiving carrot juice and the healthy control group receiving probiotic *Lactobacillus casei* were not significantly different (p-value = 0.99) (Figure 2).



*Significance of data comparing diabetic group treated with carrot juice vs. the other groups ($p = 0.001$). **Significance of data comparing diabetic group treated with mixture of *Lactobacillus casei* and carrot juice vs. the other groups ($p = 0.001$). †Significances of data comparing control group treated with carrot juice with diabetic groups ($p = 0.001$). ‡Significance of data comparing control group treated with mixture of *Lactobacillus casei* and carrot juice with diabetic groups ($p = 0.001$). CJ = carrot juice and CB = casei bacterium.

Figure 2: Changes in serum levels of glucose.

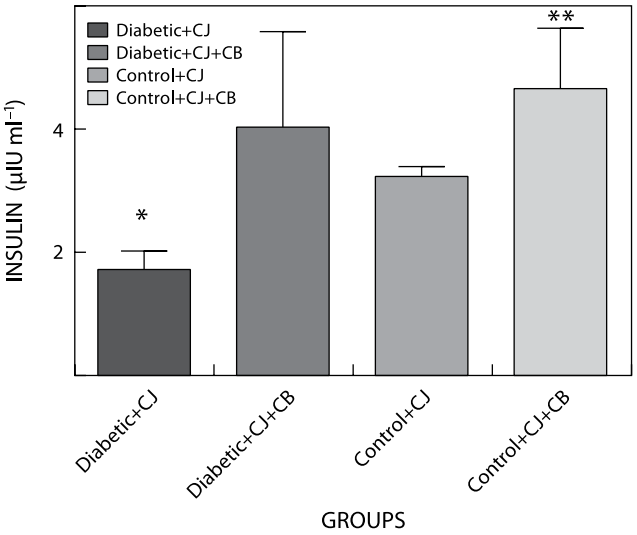
All the data from insulin, IGF-1 and C-peptide in serum were statistically analysed and these variables had a normal distribution. The results of the ANOVA test on insulin showed a significant discrepancy between the diabetic group who received carrot juice and the diabetic group treated with a mixture of probiotic *Lactobacillus casei* (p-value = 0.03), and the control group treated with a mixture of probiotic *Lactobacillus casei* and carrot juice too (p-value = 0.01); in the other groups no significant differences were seen (Figure 3).

The ANOVA test was used to consider the discrepancy among IGF-1 values in the four groups and the results showed that IGF-1 value in all four groups showed no significant difference. Thus among all groups IGF-1 is not changed (Figure 4), which means that carrot juice and probiotic *Lactobacillus casei* have no effect on diabetic and control groups.

When considering the different values of C-peptide, the ANOVA test showed significant results in three groups (p-value = 0.02 and 0.03). The results showed that except for the control group treated with a mixture of probiotic *Lactobacillus casei* and carrot juice, pairwise consideration and comparisons of the other groups showed a significant discrepancy (Figure 5). The mean ± SD of glucose, insulin, IGF-1 and C-peptide of four groups is given in Table 1.

Discussion

In 2007, Yadav *et al.* studied the effects of an anti-diabetes-containing lactobacillus Dahi produced by probiotic casei and acidophilus on rats induced with diabetes by fructose and showed that the probiotic caused a decrease in blood sugar level and glucose intolerance.[14] Further, in another study on rats with STZ-induced diabetes they showed that the blood sugar level drops significantly as a result of this probiotic. They attributed it to reinforcement of the antioxidant system with probiotics and a decrease in the destruction of beta-cells of the islets of



*Significance of data comparing diabetic group treated with carrot juice vs. diabetic group treated with mixture of *Lactobacillus casei* and carrot juice and control group treated with mixture of *Lactobacillus casei* and carrot juice ($p = 0.03$ and 0.01). **Significance of data comparing control group treated with mixture of *Lactobacillus casei* and carrot juice vs. diabetic group treated with carrot juice ($p = 0.01$). CJ = carrot juice and CB = casei bacterium.

Figure 3: Changes in serum levels of insulin.

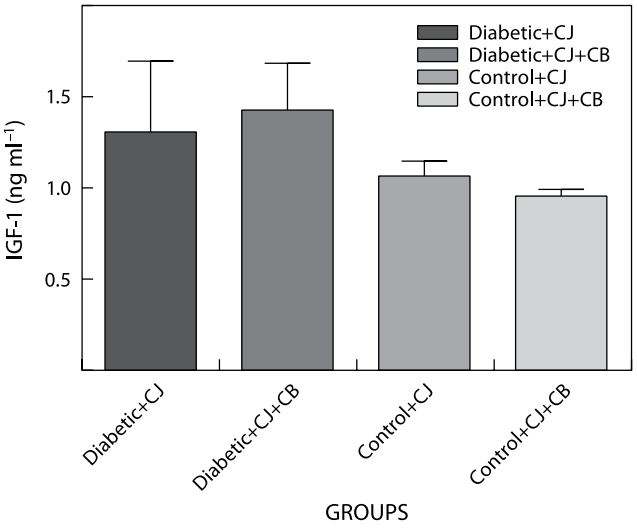
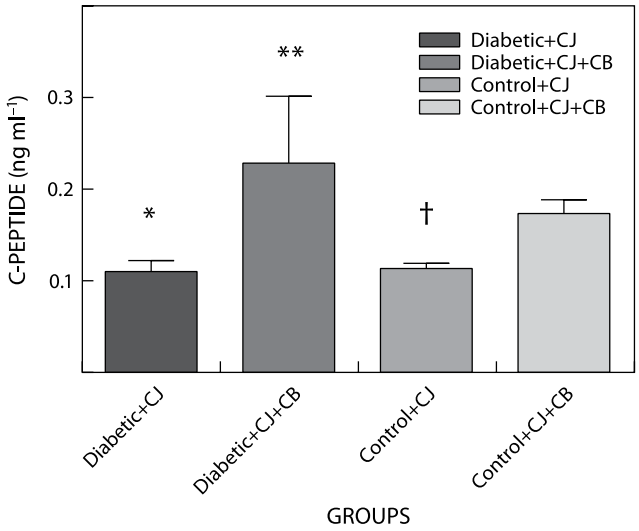


Figure 4: Changes in serum levels of IGF-1. CJ = carrot juice and CB = casei bacterium.



*Significance of data comparing diabetic group treated with carrot juice vs. diabetic group treated with mixture of *Lactobacillus casei* and carrot juice ($p = 0.005$). **Significance of data comparing diabetic group treated with mixture of *Lactobacillus casei* and carrot juice vs. diabetic group treated with carrot juice and control group treated with carrot juice ($p = 0.005$ and 0.01). †Significance of data comparing control group treated with carrot juice vs. diabetic group treated with mixture of *Lactobacillus casei* and carrot juice ($p = 0.01$). CJ = carrot juice and CB = casei bacterium.

Figure 5: Changes in serum levels of C-peptide.

Table 1: Concentration (mean ± SD) of glucose, insulin, IGF-I and C-peptide in all groups

Groups	Glucose (mg/dl)	Insulin (µlu/ml)	IGF-1 (ng/ml)	C-peptide (ng/ml)
Diabetic treated with carrot juice	456.6 ±44.9	1.72±0.3	1.3±0.3	0.11±0.01
Diabetic treated with mixture of *Lactobacillus casei* and carrot juice	238.1±27.5	4.03±1.5	1.42±0.2	0.22±0.07
Control treated with carrot juice	133.2±4.4	3.23±0.1	1.06±0.08	0.11±0.005
Control treated with mixture of *Lactobacillus casei* and carrot juice	128±11.7	4.66±0.9	0.95±0.03	0.17±0.01

p-value < 0.05.

Langerhans.[15] In 2007, Lali *et al*. proved that a Nano product containing lactobacillus causes a decrease in blood sugar of mice with diabetes through aloxone.[11] In 2009, Harissa *et al*. showed that *Lactobacillus acidophilus* causes a decrease in free radicals and reactive oxygen species (ROS). These reactive compounds cause a disorder in the nitric oxide strain of diabetic rats. Nitric oxide is an important medium in the secretion of hormones and stimulation of the immune system. In other words, *Lactobacillus acidophilus* leads to a decrease in blood sugar and adjustment of nitric oxide in rats with aloxone-induced diabetes.[16]

In 1997, Matzvky *et al.* demonstrated that *Lactobacillus casei* in KK-AY mice with genetically induced diabetes type 2 caused a decrease in blood sugar.[17] In this study the effect of probiotic *Lactobacillus casei* strain TD2 on blood sugar was investigated. The results indicated that the blood sugar in diabetic rats showed a significant decrease in comparison with a diabetic control group after consuming the probiotic for 21 days. It was also shown that the probiotic strain does not comprise a complete treatment of diabetes; however, it can be used as a controlling treatment.

Various mechanisms have been suggested for decreasing cholesterol by means of probiotics. It is likely that when the amount of lactobacillus increases in the intestine, the demand for glucose goes up and as a result leads to a decrease in released glucose in serum.[11,14] Further, because probiotics have an antioxidant trait, they prevent destruction of β-Langerhans cells

and as a result cause serum glucose to decrease.[16] The structure of intestinal microflora is effective in determining inflammation, which plays a role in diabetes. This is done when the equilibrium in the intestinal microflora is violated and gram-positive bacteria in the intestine decrease compared to gram-negative bacteria. As a result, the absorption of lipopolysaccharide and other pre-inflammation molecules and the rate of their transition to blood circulation increase. Hence, this leads to an increase in cytokine secretion, activity of macrophages, and outbreaks of inflammation. Inflammatory cytokines cause a disorder in the function of insulin receptors and as a result a resistance to insulin. Moreover, through induction of apoptosis, β-pancreases cells cause the secretion of insulin by these cells to decrease.[18–20]

In general, probiotics have been the centre of attention because of their bold effects on treating acute gastrointestinal inflammation, diarrhoea, constipation, irritable bowel syndrome, colorectal cancer, etc. Probiotics are used as food supplements but in the medical industry they can be used as auxiliary medicine with few side effects to decrease blood sugar. Researchers attribute the decrease in blood sugar to an increase in the number of lactobacilli in the intestine which consume glucose and thereby limit glucose absorption and distribution to other organs in the host.[11,14,21]

Nevertheless, more studies are required to define the exact mechanism of this process and it is likely that other lactobacilli possess this characteristic. Through more research, it may be possible to discover a simpler way to prevent and treat diabetes. The results of this study also show the efficacy of *Lactobacillus casei* in controlling blood sugar in diabetic mice. Further, the results show that probiotics in healthy mice do not cause blood sugar to decrease, which is of great importance. Therefore, it seems that the products of this probiotic can be considered an appropriate supplementary medicine for controlling blood sugar. However, it should be mentioned that various clinical studies are required to determine the accurate mechanism of this probiotic and its daily consumption.

Compliance with ethical standards

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. What about animal ethics approval?

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Received: 07-01-2017 Accepted: 08-09-2017