Effect of Lactic Acid Bacteria on the Regulation of Blood Glucose Level in Streptozotocin-induced Diabetic Rats

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ABSTRACT

To identify the treatment effect of lactic acid bacteria for diabetes, the treatment effects of a single administration of acarbose (a diabetes treatment drug) or lactic acid bacteria, and the mixture of acarbose and lactic acid bacteria on diabetes in a type 1 diabetes animal model, were studied. In this study, streptozotocin was inoculated into a Sprague-Dawley rat to induce diabetes, and sham control (Sham), diabetic control (STZ), STZ and composition with live cell, STZ and composition with heat killed cell, STZ and composition with drugs (acarbose) were orally administered. Then the treatment effect on diabetes was observed by measuring the body weight, blood glucose, and serum lipid. For the histopathological examination of the pancreas, the Langerhans islet of the pancreas was observed using hematoxylin and eosin staining, and the renal cortex, outer medullar, and inner medullar were also observed. The induced diabetes decreased the body weight, and the fasting blood glucose level decreased in the lactic-acid-bacteria-administered group and the mixture-administered group. In addition, the probiotic resulted in the greatest decrease in the serum cholesterol level, which is closely related to diabetes. Also, the hematoxylin and eosin staining of the Langerhans islet showed that the reduction in the size of the Langerhans islet slowed in the lactic-acid-bacteria-administered group. The histopathological examination confirmed that the symptoms of diabetic nephropathy decreased in the group to which viable bacteria and acarbose were administered, unlike in the group to which dead bacteria was administered. The mixture of lactic acid bacteria and acarbose and the single administration of lactic acid bacteria or acarbose had treatment effects on the size of the Langerhans islet and of the kidney histopathology. Thus, it is believed that lactic acid bacteria have treatment effects on diabetes and can be used as supplements for the treatment of diabetes.

(Key words : Actic acid bacteria, Streptozotocin, Diabetic rats, Blood glucose level, Acarbose)

INTRODUCTION

Diabetes is a disease category that involves various factors characterized by a high blood glucose level and the accompanying metabolic dysfunction. It is caused by an absolute or relative deficiency of insulin or the decreased effect of insulin on the target cell due to increased insulin tolerance (Beckman *et al.*, 2002). Diabetes is classified into type 1 and type 2. In type 2 diabetes, thirst, a large amount of urine, increased appetite, and loss of body weight occur (Lupi *et al.*, 2008). Also, type 2 diabetes is related to decreased metabolism due to a large amount of active oxygen and oxidative stress (Hayden *et al.*, 2005).

In a study on the etiology of diabetes, streptozotocin (STZ) was inoculated into a rat to induce diabetes (Rakieten *et al.*, 1963; Kim, 2005; Yoon and Son, 2009). The STZ that was inoculated into the rat moved to the pancreatic β cell, attacked the mitochondria, and decreased the ATP synthesis of the mitochondria. Consequently, the ADP level in the mitochondria increased (Rakieten *et al.*, 1963). STZ is known to induce experimental diabetes by selectively attacking the pancreatic islet β cell (Junod *et al.*, 1967 and Cantenys *et al.*, 1981). The increased ADP in the β cell is converted into hypoxanthine and xanthine via catabolism, and subsequently, into uric acid and superoxide anion by xanthine oxidase, which exists in a high concentration in the β cell.

Moreover, as STZ directly activates xanthine oxidase, the catalytic effect of a high concentration of xanthine oxidase increases, and subsequently, the production of superoxide anion is accelerated. A study reported that STZ-induced diabetes developed when the increased amount of the superoxide anion was metabolized and the β cell was subsequently damaged or the increased amount of the superoxide anion itself directly damaged the β cell (Kawada *et al.*, 1992). Besides, type 2 diabetes is known to be related to various cases of decreased metabolism caused by a large amount of active oxygen and oxidative stress (Babu *et al.*, 2004).

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Acarbose, one of the many drugs that are being used to treat diabetes, may induce flatulence because it inhibits carbohydrate-digesting enzymes, after which the undigested carbohydrates are broken down by harmful intestinal bacteria into carbon dioxide and hydrogen (Clissold *et al.*, 1988). Many studies have been conducted to overcome this side-effect of acarbose. Meanwhile, no studies have been conducted yet to find a way to treat diabetes by administering lactic acid bacteria or mixed lactic acid bacteria, among the many possible diabetes treatment foods and food supplements. Previous studies reported various health promotion effects of lactic acid bacteria, such as lowering of the intestinal pH by producing lactic acid as a metabolite and consequently suppressing the proliferation of harmful bacteria (Perdigon *et al.*, 1990); influencing the intestinal microbial fauna (Shahani *et al.*, 1980); inhibiting the development of intestinal disease by removing intestinal toxic matter and strengthening the specific or non-specific immunologic function (Shida *et al.*, 1998); lowering the serum cholesterol level (Chiu *et al.*, 2006) and accentuating the hepatic function (Kim *et al.*, 2007), apart from its anticancer effect (Kato *et al.*, 1994), antioxidative effect (Hochgrafe *et al.*, 2008) and reduction of lactic acid intolerance (Ouwehand *et al.*, 2002). Thus, the administration of vital or dead lactic acid bacteria or mixed lactic acid bacteria is expected to reduce the side-effects of acarbose, such as flatulence, by suppressing the growth of harmful microorganisms.

The purpose of this study was to identify the efficacy of lactic acid bacteria in the treatment of diabetes when administered in combination with acarbose, by observing the blood glucose level and size of pancreatic beta in STZ-induced diabetic rats, and on the serum insulin level.

MATERIALS AND METHODS

Animals and Experiment Design

Male Sprague-Dawley (SD) rats (six weeks old, 200~230 g each), which were procured from Joong-ang Laboratory Animals Co., Ltd., were acclimated for one week and then randomly assigned to the following six groups (with eight rats in each group): sham control (sham), diabetic control (STZ), STZ and composition with live cell (live cell), STZ and composition with prebiotic (heat killed cell), STZ and composition with drugs (acarbose), and STZ and combined treatment with lactic acid bacteria and acarbose (Mixture). The rats were raised at a temperature of 23±1℃, a humidity of 55± 10%, and exposed to 12-hr light (07:00~19:00), after which they were given free access to the test feed and water. Vital and dead *Lactobacillus paracasei* (KCTC13-413), *Lactobacillus rhamnosus* (KCTC3929), and *Streptococcus thermophilus* (KCTC3927) were mixed (1.0E+08 cfu each) and orally administered once daily. Acarbose was administered at a 50 mg/kg dose. The rats were made to fast for 16 hrs to induce diabetes, and 50 mg/kg of STZ, which was dissolved in a 0.01 M citrate buffer with a pH of 7.4, was injected into the tail vein of the rat. Forty-eight hrs later, the rats were made to fast for 16 hrs to confirm the induction of diabetes, and only those that were found to have a blood glucose level of 300 mg/dl or more using a blood glucose measuring device (ACCU-CHEK Active: Roche, Germany) were considered diabetic rats and were used in the experiment. The body weight was measured immediately before the administration of STZ, and then each week immediately before fasting 30 days after the administration of the sample. Upon completion of the experiment, the rats were sacrificed after anesthetization, and a blood sample was collected from the abdominal artery. The serum was isolated from the blood sample, and then the serum lipid was analyzed using a FUJIFILM DRI-CHEM 4000i biochemical analyzer (Kanagawa, Japan). This study was approved by the Animal Experiment Ethics Committee (CBTA-006) and complied with the Regulations on Animal Management. All the experiment protocols in this study were reviewed and approved by the Animal Care and Use Committee of Cellbiotech Co., Ltd. of Korea.

Histopathological Examination of the Pancreas

After the rats were sacrificed by anesthetization, a blood sample was collected. Then after perfusion fixation was performed, the excised pancreas was fixed in 10% formalin. The fixed pancreas was cut thin, fixed again in 10% formalin at room temperature for 24 hrs, and washed with tap water for one hr. The washed tissue was loaded onto an automatic tissue processor (Hypercenter XP, Shandon, England). After the process of dehydration, clearing, and infiltration in the tissue processor, the tissue was embedded in paraffin and then its histologic section was cut off using microtome. After the process of paraffin removal and dehydration, the histological section was stained via hematoxylin-eosin staining (H&E staining) and observed under an optical microscope.

Statistics Analysis

The data was processed with Graphpad PrismTM 4.0. The mean value and standard deviation was calculated in a group, and the significance was compared with student *t*-test ($p<0.05$) between groups.

RESULTS AND DISCUSSION

Analysis of the Body Weight and Glucose

Table 1. Patterns of body weight in control and STZ-induced diabetic rats at each stage

Body weight (g)	Pre-injection	3 days	9 days	15 days	21 days	27 days
Sham	205± 6.4	224±10.2	268±18.1	290±19.2	307±20.7	312±24.9
Control	175±12.2	178± 6.5	180±18.6	173±14.4	159±20.2	165±19.7
Probiotic	174±20.0	179±16.7	185±19.1	181±23.1	180±22.9	190±27.2
Prebiotic	184± 4.7	188± 7.9	191±11.8	181± 8.3	180± 5.0	180± 3.1
Acarbose	183± 8.6	193±12.9	185±19.3	177±20.8	173±20.2	179±24.6
Mixture	182± 7.9	186± 4.5	194± 7.6	195±11.5	192±14.8	205±23.2

To determine if there was a decrease in the body weight, which is characteristic of type 1 diabete, the body weight of the STZ-induced diabetic rats was measured every six days up to 27 days. Consistent with the chacteristics of type 1 diabetic rats, the body weight inrcreased only in the sham group, and not in the STZ-induced diabetic groups (Table 1). The average body weight of the individual rats in the sham groups was heavier than that of the individual rats in the STZ group, which suggests that type 1 diabetes influenced the decrease in the body weight. Also, the fasting blood glucose level did not significantly differ between the groups until after two weeks, following which it significantly increased (p<0.05) to 450 mg/dl at 27 days in the STZ group and to 350 mg/dl in the mixture group. Thus, it is believed that mixture of lactic acid bacteria and acarbose can have a therapeutic effect on the loss of the body weight and the increase in the blood glucose level (Fig. 1).

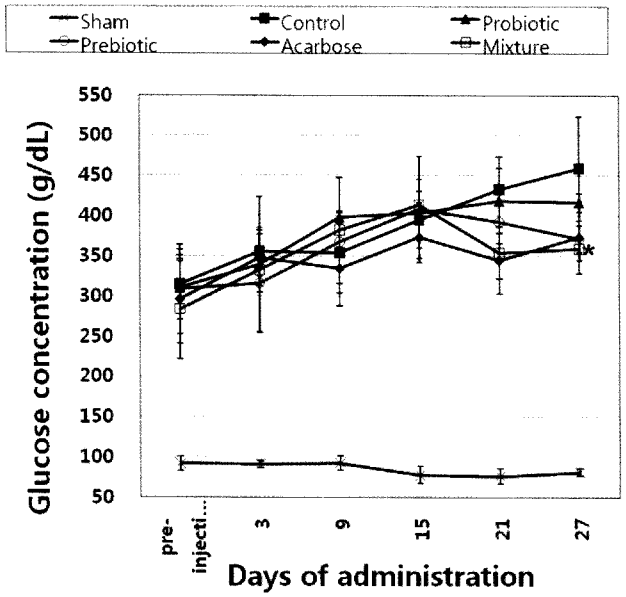

Fig 1. Patterns of glucose concentraion in control and STZ-induced diabetic rats at each stage. Sham (×): sham injection, Control (■): streptozotocin (STZ)-induced diabetic rats, Probiotic (▲): probiotic (10^8 cfu) in STZ-induced diabetic rats, Prebiotic (○): Prebiotic (10^8 cfu) in STZ-induced diabetic rats, acarbose (◆): Acarbose (50 mg/kg) in STZ-induced diabetic rats, Mixture (□): mixture (probiotic 10^8 cfu + prebiotic 10^8 cfu + acarbose 50 mg/kg) in STZ- induced diabetic rats. The error bars indicate S.D. *Mixture was statistically different from control, as determined by t-test (nonpaired) with GraphPad Software (p<0.05).

Lactic acid bacteria are expected to help improve body weight loss in diabetes. Also, the blood glucose level, which is an important parameter of diabetes, sharply decreased 15 days after the administration of the sample in all the treatment groups, except for the STZ group. The treatment effect of lactic acid bacteria on diabetes in the diabetic rats started 15 days after their administration, and the blood glucose level in the Acarbose and mixture groups considerably decreased compared with that in the STZ group at 21 days and 27 days, respectively. Also, it slightly decreased in the live cell and prebioticgroups compared with that in the STZ group. Thus, the treatment effect of lactic acid bacteria was higher in the acarbose and mixture groups than in the live cell group in terms of the blood glucose level. The serum cholesterol level was lower in the live cell group than in the STZ group. Taken together, these results suggest the possibility that lactic acid bacteria can decrease the blood glucose and serum cholesterol levels in diabetic animal models. To elucidate the treatment effect of lactic acid bacteria on diabetes, a study period of more than 27 days is required.

Analysis of the Change in the Serum Glucose after Feeding

In diabetic patients, postprandial blood glucose sharply increases, and its control is known to be critical. acarbose, which was used in this study, regulates the blood glucose level by inhibiting the enzyme that is involved in the absorption of glucose. Thus, diabetic rats were made to fast for 12 hrs, orally given the sample, and fed for one hr. Then the blood glucose level was measured at one-hour intervals for 3 hrs (thrice) to examine the change in the level with the administration of the sample. In the STZ group, the postprandial blood glucose level increased and remained high for 3 hrs after the feeding. In the probiotic and prebiotic groups, the postprandial blood glucose level increased to a le-

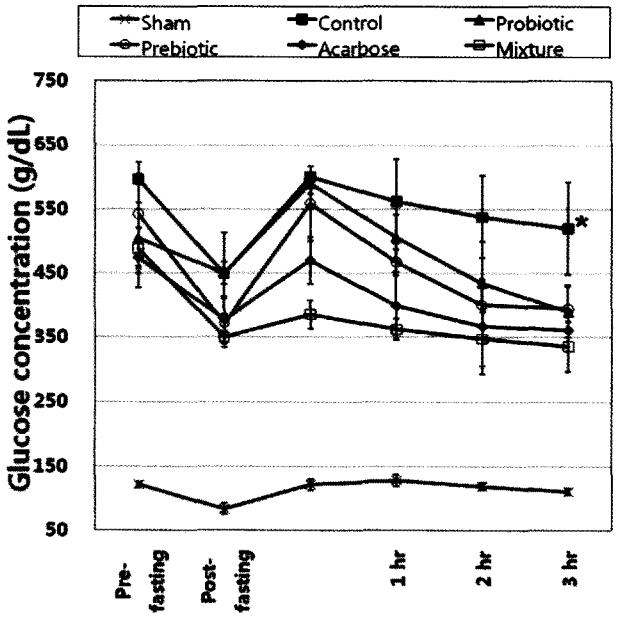

Fig. 2. Analysis of glucose concentraion in control and STZ-induced diabetic rats after feeding. Sham (×): sham injection, Control (■): streptozotocin (STZ)-induced diabetic rats, Probiotic (▲): Probiotic (10^8 cfu) in STZ-induced diabetic rats, Prebiotic (○): Prebiotic (10^8 cfu) in STZ-induced diabetic rats, Acarbose (◆): Acarbose (50 mg/kg) in STZ-induced diabetic rats, Mixture (□): mixture (probiotic 10^8 cfu + prebiotic 10^8 cfu + acarbose 50 mg/kg) in STZ-induced diabetic rats, A: body weight, B: glucose concentraion. The error bars indicate S.D. *Mixture was statistically different from control, as determined by *t*-test (nonpaired) with GraphPad Software ($p<0.05$).

vel similar to that in the diabetic group, but sharply decreased until 3 hrs after the feeding. Contrary to this, in the acarbose and mixture groups, the postprandial blood glucose level was significantly lower ($p<0.05$) than in the STZ group and remained at 200 mg/dl for 3 hrs after the feeding, which suggests that the regulation of the postprandial blood glucose level was influenced (Fig. 2).

Comparison with the Histology of the Langerhans Islet

Considering that in type 1 diabetes, β-cells, which secrete insulin and mostly make up the Langerhans islet in the pancreas, are destroyed and thus, blood glucose is not regulated, the size of the Langerhans islet was observed using hematoxylin and eosin staining. In the STZ group (Fig. 3C and D), the size of the Langerhans islet was considerably reduced compared with that in the sham group (Fig. 3A and B). In the live cell (Fig. 3E and F) and prebiotic (Fig. 3G and H) groups, the size of the Langerhans islet slightly increased compared with that in the STZ group. In the acarbose (Fig. 3I and J) and mixture (Fig. 3K and L) groups, the size of the Langerhans islet slightly increased compared with that in the STZ group. Considering these results all together, the size of the Langerhans islet increased in the STZ-induced diabetic rats when lactic acid bacteria, acarbose, or a mixture of both was administered.

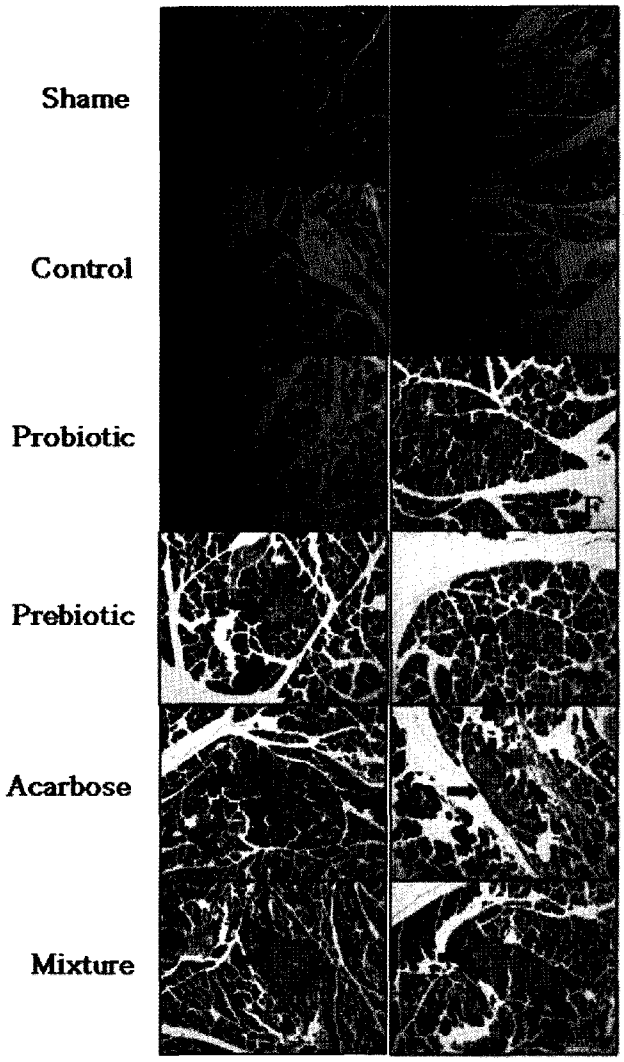

Fig. 3. Langerhans islets of pancreas, hematoxylin-eosin staining. Sham. (A and B), Control: (C and D), Probiotic: (E and F), Prebiotic: (G and H), Acarbose: (I and J), Mixture: (K and L). The arrow at the right indicates Langerhans islets of pancreas.

Although the postprandial blood glucose level, an important indicator of diabetes, increased to a level similar to that of the STZ group, it drastically dropped until 3 hrs after the feeding. Moreover, consistent with the results of the study of Leonhardt *et al.* (1994), who reported that acarbose lowered the blood glucose level by suppressing the function of carbohydrates-digesting enzymes, the increase in the blood glucose level in the acarbose and mixture groups was lower than that in the other groups, even when the rats were fed. Particularly, it is considered that the lower increase rate of the blood glucose level in the acarbose and mixture groups was due to the synergy effect of the lactic acid bac-

teria in the regulation of the postprandial blood glucose level. The blood glucose level decreased to a certain level in all the groups until 3 hrs after the feeding, except for the STZ group, which indicates that the administration of lactic acid bacteria alone can have a treatment effect on diabetes.

The recovery of type 1 diabetes was determined by observing the size of the Langerhans islet, which controls insulin secretion. As shown in Fig. 3, the size of the Langerhans islet in all the treatment groups was bigger than that in the STZ group. It is believed that among the treatment groups, the treatment effect in terms of the recovering of the size of the Langerhans islet in the mixture group was higher than in the live cell and prebioticgroups. Furthermore, the administration of acarbose in combination with lactic acid bacteria is expected to have a better treatment effect in terms of the recovery of the function of insulin secretion regulation than the single administration of acarbose. Considering that increased blood glucose and serum lipid levels lead to diabetic nephropathy, the kidneys of the diabetic rats were examined.

Investigation of the Histology in the Pancreas

To confirm the presence of diabetic nephropathy, a possible complication of diabetes, the cortex and the outer and inner medullar of the kidney were separately stained. Besides, in diabetic nephropathy, various histological changes are known to occur such as glomerular basement membrane thickening, tubular basement membrane thickening, mesangial expansion, interstitial expansion, tubular dilation, nodular glomerulosclerosis, and afferent and efferent arteriolar hyalinosis (Lane *et al.*, 1991). In this study, no such histological changes occurred in the cortex of the STZ group (Fig. 4D, E and F). In the medullar, however, such histological changes as interstitial expansion and tubular dilation were observed. In the live cell, heat killed cell, acarbose, and mixture groups, such histopathological changes as interstitial expansion and tubular dilation almost disappeared. Therefore, it is considered that the treatment effect on diabetic nephropathy was higher in the live cell and Acarbose groups than in the prebioticgroup (Fig. 4). Consequently, histopathological changes such as interstitial expansion or tubular dilation rarely occurred in the live cell, heat killed cell, acarbose, and mixture groups, unlike in the STZ group. Thus, lactic acid bacteria or a mixture of acarbose and lactic acid bacteria tends to treat diabetic nephropathy.

Analysis of Cholesterol in the STZ-induced Diabetic Rats

Serum cholesterol, a serum lipid, may induce arterial sclerosis and is known to affect the development of diabetic nephropathy, a complication of diabetes (Wakeel *et al.*, 2009). To measure the serum cholesterol level, a

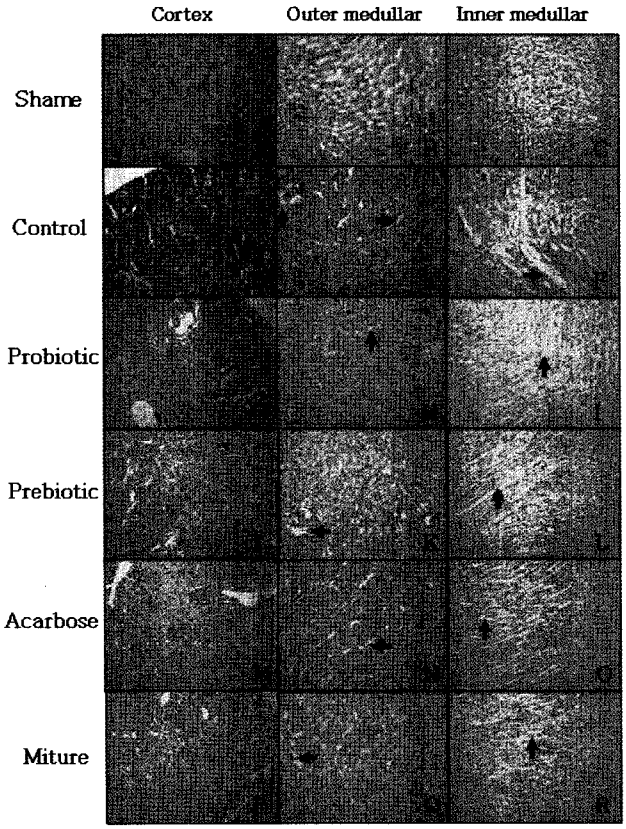

Fig. 4. Histopathology of kidney, hematoxylin-eosin staining. Sham: (A and B), Control: (C and D), Probiotic: (E and F), Prebiotic: (G and H), Acarbose: (I and J), Mixture: (K and L). The arrow indicates the interstitial expansion or dilated tubule.

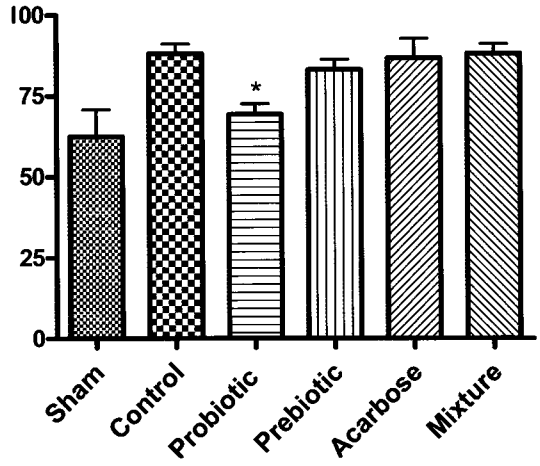

Fig. 5. Analysis of cholesterol concentraion in control and STZ-induced diabetic rats. Sham: sham injection, Control: streptozotocin (STZ)-induced diabetic rats, Probiotic: probiotic (10^8 cfu) in STZ-induced diabetic rats, Prebiotic: prebiotic (10^8 cfu) in STZ-induced diabetic rats, acarbose: Acarbose (50 mg/kg) in STZ-induced diabetic rats, Mixture: mixture (probiotic 10^8 cfu + prebiotic 10^8 cfu + acarbose 50 mg/kg) in STZ-induced diabetic rats. The error bars indicate S.D. *Probiotic was statistically different from control, as determined by *t*-test (nonpaired) with GraphPad Software ($p< 0.05$).

blood sample was collected from the abdominal vein of the rats to which the sample was administered for 27 days. In the STZ group, the serum cholesterol level was 88 mg/dl, and in the acarbose group, 86.7 mg/dl, without significant difference between the two groups. This result is consistent with that reported by Leonhardt *et al.* (1994), in which acarbose did not affect the serum cholesterol level. On the contrary, the serum cholesterol level in the live cell group was 69 mg/dl, about 20% lower than in the STZ group, which suggests that lactic acid bacteria can decrease the STZ-induced increase in the serum cholesterol level (Fig. 5).

Taken together, lactic acid bacteria and the mixture of lactic acid bacteria made of vital or dead *Lactobacillus paracasei* (KCTC13413), *Lactobacillus rhamnosus* (KCTC3929), and *Streptococcus thermophilus* (KCTC3927) were shown to have a treatment effect on diabetes, and the mixture of acarbose and lactic acid bacteria is expected to have a synergy effect on the treatment of such disease.

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