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LETTERS

Lactobacillus casei and *Bifidobacterium bifidum* reduces postprandial hyperglycaemia, inhibits α-glucosidase activity and improve histology of pancreatic islets in streptozotocin induced diabetic rats

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ABSTRACT

Lactobacillus and *Bifidobacteria* are generally accredited as potential anti-diabetic agents, but the mechanism of action is still not clear. This study aimed to investigate the result of administration of *Lactobacillus casei and Bifidobacterium bifidum* on α-glucosidase enzyme, postprandial blood glucose level, and histological changes that occur in the pancreatic β-cells in streptozotocin (STZ (50 mg/kg body weight)) induced diabetic rats. The experimental diabetic rats were treated with a single dose of *L. casei* and *B. bifidum* alone and combination for 28 days. The diabetic animals of a positive control group were treated with the standard hypoglycaemic drug, acarbose (10mg/kg body weight). Maltose and starch tolerance tests were performed. Treatment demonstrated significant hypoglycemic activity, lower postprandial blood glucose levels induced by maltose and starch loading in diabetic rats. The results demonstrated that *L. casei* and *B. bifidum* had α-glucosidase inhibitory activity and can reduce blood glucose level observed from the maltose and starch tolerance test. Histological analysis showed treatment prevented the function of pancreatic islets and regenerate degenerated cells.

Keywords: α-glucosidase, Bifidobacterium bifidum, Diabetes, Lactobacillus casei, postprandial hyperglycaemia

INTRODUCTION

Diabetes mellitus is a complex metabolic disorder characterized by constant hyperglycaemia due to defect of insulin secretion or insulin action. Continuous hyperglycaemia condition may be associated with a broad range of complications like, retinopathy, neuropathy, nephropathy and risk of cardiovascular disease.[1] Diabetes is increasing at an alarming rate due to environmental factors and changes in lifestyle. According to International Diabetes Federation (IDF), currently, the worldwide prevalence of diabetes is 463 million and is predicted to reach 578 million in 2030 and 700 million in 2045 if proper preventive programs are not established.[2] Management of diabetes can be done by exercise, diet and use of various hypoglycaemic agents but long-term use of these synthetic anti-diabetic drugs has some serious adverse effects like hypoglycaemia, flatulence, weight gain, and bloating.[3,4] Anti-diabetic drugs from natural sources seem to be more desirable as novel hypoglycaemic agents.[5,6] Recent work is favoring natural

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and safe agents those help to increase the function of β-cells and have significant glycemic control.

Nutritional supplements like probiotics provide a natural source of oral hypoglycaemic agents and these are safe to use with minimal or no adverse effect. Probiotics are considered a most popular functional food. Probiotics treatment has relatively low costs compared to synthetic anti-diabetic agents. Recently many researchers are focusing on the use of probiotics because of their beneficial health effects. Previous studies have reported potential health benefits of lactic acid bacteria (LAB) and *Bifidobacteria*, such as anti-diabetic activity, anti-microbial activity, enhance the immune system and improve gut microbiota.[7] Zeng et al reported α-glucosidase inhibitory activity of LAB and suggested that LAB might serve to alleviate the effects of hyperglycaemia.[8] Panwar et al also (2014) reported that Lactobacillus strains are present in the human gut and have α-glucosidase inhibitory activity.[9] Post-prandial hyperglycemia is effectively controlled by delaying digestion and absorption of carbohydrates by using inhibitors of α-glucosidase such as acarbose, voglibose and miglitol.[10] Enzyme α-glucosidase is found in the intestine and is responsible for the digestion of complex oligosaccharides into simple monosaccharides. Now-a-day incretin-based therapy and inhibitors of α-glucosidase are used to control hyperglycaemic conditions. The result of our previous study indicated that *L. casei* and *B. bifidum* treatment has significant anti-hyperglycemic, hypolipidemic and antioxidant activity in diabetic Wistar rats.[11] This study aims to investigate the inhibitory activity of *L. casei* and *B. bifidum* on carbohydrate hydrolyzing enzyme α-glucosidase and the role of treatment against STZ-induced pancreatitis.

MATERIALS AND METHODS

Chemicals and Reagents

STZ was purchased from Sigma-Aldrich, St Louis, MO, USA. De Man, Rogosa and Sharpe (MRS) broth, MRS Agar, acarbose, L-cysteine, maltose, starch were obtained from HiMedia Laboratories (Mumbai, India). Blood glucose was estimated by using Accucheck one touch glucometer. The α-glucosidase was estimated by using a standard kit procured from Sigma, St. Louis, MO, USA.

Bacterial strain

Pure strains of *Lactobacillus casei* NCDC017 and *Bifidobacterium bifidum* NCDC231 were acquired from the National Collection of Dairy Culture, National Dairy Research Institute (N.D.R.I.), Karnal, India.

Animals

Male Wistar rats (160-220 g body weight) were purchased from the animal research division, Defence Research & Development Establishment (D.R.D.E.) Gwalior, Madhya Pradesh, India. The study was approved by the Institutional Animal Ethical Committee (I.A.E.C.) (Reg.No.-BU/PHARMA/IAEC/a/16/12). The animals were kept in an animal house under the standard environmental conditions of temperature 22±2 ^{0}C and humidity 55±5% with a 12 h light-dark cycle. The rats were kept in plastic cages with husk bedding and the cages were cleaned and changed daily. The animals were provided RO water and a normal pellet diet ad libitum. Before starting the experiment the rats were acclimatized for one week.

Induction of diabetes

Diabetic rats were induced by single dose of freshly prepared STZ solution (50 mg/body weight) in 0.1 M citrate buffer (pH 4.5) to overnight fast animal intraperitoneally. Fasting blood glucose levels were measured after 96 hours using glucometer. Blood glucose level was taken regularly till stable hyperglycaemia condition achieve. Rats with fasting blood glucose level higher than 250 mg/dl were considered to be diabetic.

Dosing preparation

The pure strain of *L. casei* and *B. bifidum* were received from collection center in lyophilized form, revived in MRS broth medium. About 1 mL of inoculums were serially diluted six times in sterile distilled water in sterile condition and 100 µL of sixth dilution was inoculated on MRS agar plate by spreading method in anaerobic condition in an anaerobic system (Anaero Gas Pack, LE002. HiMedia, India) at 37 °C for 48 h. After incubation, 50-60 colonies appear in each plate that further used to prepare dose for experimental rats. The concentration of last dilution was approximately ~56×10^7 cfu/mL. A single colony was picked up from culture plate by using sterile loop and mixed well using micropipette in 1 mL autoclaved distilled water. All doses were prepared in laminar air flow in aseptic conditions.

Experimental design

All the rats were randomly divided into six experimental groups and each group consisted of six animals. All groups of animals were subjected to given the following treatments during 28 days. (Table 1):

Table 1: Experimental design of animal.

S.N.	Group	Name of group	Treatment
1.	Group I	Healthy control	Single daily dose of sterile distil. water 1mL
2.	Group II	Diabetic control	Single daily dose of sterile distil. water 1mL
3.	Group III	*L. casei* treated diabetic	Single daily dose of *L. casei*
4.	Group IV	*B. bifidum* treated diabetic	Single daily dose of *B. bifidum*
5.	Group V	*L. casei* and *B. bifidum* combination treated diabetic	Single daily dose of *L. casei* and *B. bifidum*
6.	Group IV	Acarbose treated diabetic	Single daily dose of acarbose (10 mg/kg b.w.)

PPG, FBG and food intake

The fasting blood glucose (FBG) and postprandial blood glucose (PBG) were monitored on 0, 7th, 14th, 21st and 28th days by using a one-touch glucometer. Blood sample obtained from the tail vein that was first cleaning by ethyl alcohol cotton swab and also, the second drop of blood was used after removing the

first drop of blood. Food intake by experimental rats was measured weekly.

Maltose and starch tolerance test

Animals were fasted overnight and administered maltose/starch (2 g/kg body weight) orally dissolved in 1 mL of distilled water in combination with regular treatment. Blood samples were collected from the tail vein at 0, 30, 60, 90 and 120 minutes. Blood glucose level was analyzed by using Accucheck one-touch glucometer.

α-glucosidase inhibitory activity

After the termination of the experiment, rats were sacrificed by giving ether anesthesia; the intestine was removed, flushed with ice-cold physiological saline and processed for enzyme analysis. α-glucosidase activity assayed by the method of Liu *et al.*[12] Briefly, the intestinal part immediately below the duodenum and cecum was cut, homogenized in 12 ml of maleate buffer (100 mM, pH 6.0), centrifuged at 3,000×g for 10 min, the supernatant was taken and used as crude enzyme solution. The activity of intestinal α-glucosidase was measured by α-glucosidase activity assay kit (Sigma, St. Louis, MO, USA-MAK 123) using Thermo Scientific Varioskan Flash Spectral Scanning Multimode Reader.[13]

Histopathology assessment of Pancreases

Pancreatic tissues were removed from sacrificed animals, washed with ice-cold phosphate buffer saline and stored in 10% formalin. Tissues were processed for histological examination and stained with hematoxylin and eosin**.** Histological observations were performed using Olympus Penta head microscope.

Statistical analysis

All the data were presented as mean ± standard error mean (SEM) and analyzed by using statistical software called statistical package for social sciences (SPSS version 20.0, IBM). All analysis were calculated by one-way ANOVA followed by Tukey's multiple range post hoc tests, with levels of significance of $p<0.05$.

Result and Discussion

Effect of treatment on FBG and PBG

The FBG and PBG are two noticeable parameters of diabetes. Figure 1 shows the weekly FBG and PBG levels of experimental animals during the study. Compared to the normal healthy rats, diabetic rats showed a higher levels of FBG and PBG. Treatment with both probiotic strains alone and in combination to diabetic rats caused a significant reduction of blood glucose levels. Among all treated groups, the group treated with the combination of both bacteria and acarbose showed a significant decrease in FBG and PBG levels as compared to the diabetic group. The present study revealed that oral administration of *L. casei* and *B. bifidum* displayed a remarkable anti-hyperglycemic effect in STZ-induced diabetic rats. The treatment with the combination of both bacteria was the most effective dose to diabetic animals when comes to the treatment with probiotics. The dose showed approximately the same effect as that of the standard anti-diabetic drug acarbose. Our hypothesis was these strains exert an anti-diabetic effect which was supported by the results obtained in the present study. Treatment could effectively decrease FBG and PBG levels, which is consistent with previous studies.[12] Many studies have reported that probiotics showed potential anti-diabetic effects with high efficiency and few side effects.[14,15]

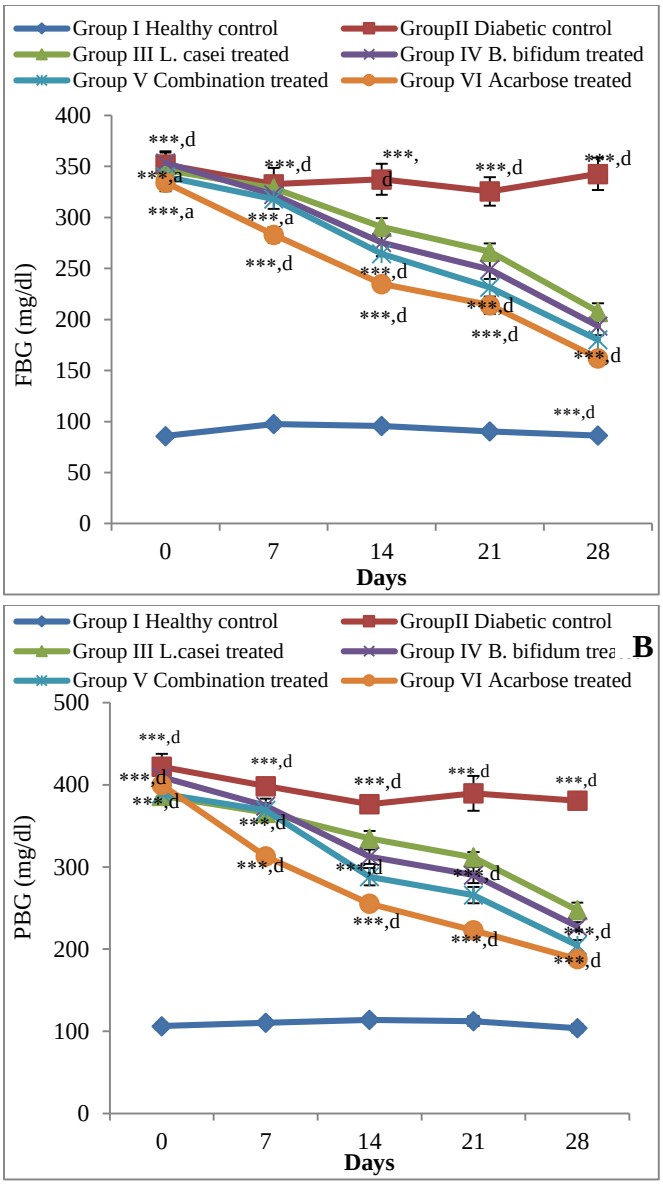

Figure 1. Effect of treatment on FBG (A) and PBG (B) levels. Result were presented as mean ± SEM (n=6 animal/group); $p > 0.05$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to healthy control, $^{a}p > 0.05$, $^{b}p < 0.05$, $^{c}p < 0.01$, $^{d}p < 0.001$ compared to diabetic control.

Effect of treatment on food intake

The changes in food intake are displayed in Figure 2. The food intake in the diabetic group was significantly higher ($p<0.05$) than in other experimental groups. Food uptake was suppressed in experimental animals that were treated with anti-diabetic standard drug acarbose, *L. casei* and *B. bifidum* alone and in combination. Since acarbose is a potent inhibitor of α-glucosidase enzyme and many studies reported that *Lactobacillus* and *Bifidobacteria* possess α-glucosidase inhibitory activity, therefore, during treatment, reduction in food intake was

observed due to the carbohydrate digestion in the intestine and delay in the digestion of carbohydrate that further mediates and improves hyperglycemia. Li et al reported that berberine treatment significantly reduced food intake in STZ induced diabetic rats.[12]

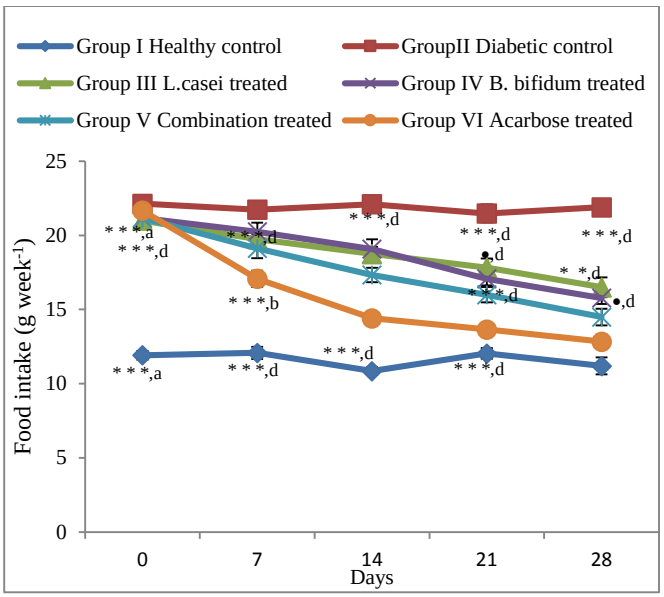

Figure 2. Effect of treatment on food intake in different experimental groups. Result were presented as mean ± SEM (n=6 animal/group); $p > 0.05$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to healthy control, $^{a}p > 0.05$, $^{b}p < 0.05$, $^{c}p < 0.01$, $^{d}p < 0.001$ compared to diabetic control.

Our previous study showed that oral administration of *L. casei* and *B. Bifidum* increases the level of gut-derived incretin hormones, glucagon like peptide-1, that might affect food intake because it stimulates satiety.[16]

Effect of treatment on carbohydrate challenge test

Oral maltose and starch tolerance test were performed in healthy rats and diabetic rats. It was found that glucose levels increased sharply in diabetic rats, maximum at 60 min after oral administration of maltose and starch. Treatment with the combination of *L. casei* and *B. bifidum* can significantly reduce postprandial blood glucose levels, induced by maltose or starch loading in diabetic rats. Diabetic animals treated with both *L. casei* and *B. bifidum* have slightly higher glucose levels than the diabetic rats treated with the standard anti-diabetic drug acarbose. On the other hand, negative control diabetic rats showed a very high levels of glucose after the maltose or starch tolerance test (Figure 3). One of the reasons for decreased glucose levels in experimental animals treated with the combination of both probiotic strains could be due to α-glucosidase inhibitory activity of *L. casei* and *B. bifidum* due to which digestion and absorption of maltose or starch in the intestine was delayed. The standard anti-hypoglycaemic drug, acarbose also suppressed the postprandial blood glucose level after maltose or starch load.

Effect on α-glucosidase enzyme

The anti-diabetic activity of *L. casei* and *B. bifidum* was further supported by the inhibitory action of the selected probiotic strains on carbohydrate hydrolyzing enzyme α-glucosidase. The results showed that intestinal α-glucosidase activity was significantly ($p< 0.01$) increased in diabetic control rats as compared to non-diabetic healthy rats. However, treatment of diabetic group with acarbose and a combination of *L. casei* and *B. bifidum* significantly decreased ($p<0.01$) the enzyme activity in diabetic experimental rats (Figure 4). The animal groups treated with *L. casei* and *B. bifidum* alone were also able to inhibit α-glucosidase enzyme activity. Post-prandial hyperglycemia may be managed or controlled by inhibiting the activity of intestinal α-glucosidase.

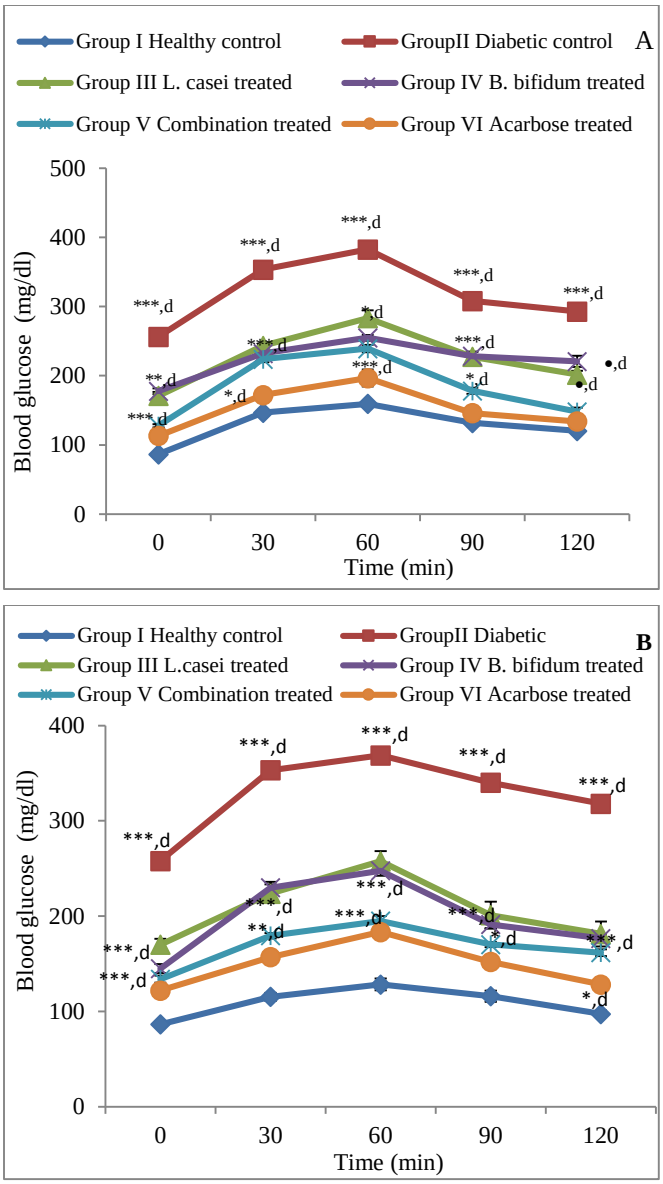

Figure 3. Effect of treatment on glucose excursions in rats after challenges with maltose (A) and starch (B). Rats were orally gavage with either (a) maltose (2g/kg) or (b) starch (2 g/kg) and glucose response was monitored at 0, 30, 60, 90 and 120 min. Result were presented as mean ± SEM (n=6 animal/group); $p > 0.05$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to healthy control, $^{a}p > 0.05$, $^{b}p < 0.05$, $^{c}p < 0.01$, $^{d}p < 0.001$ compared to diabetic control.

Recently α-glucosidase inhibitors from natural sources have gradually become a safe and promising therapy for the

management of postprandial hyperglycemia. A previous study showed that LAB produced exopolysaccharides was able to inhibit the α-glucosidase enzyme.[17] Probiotic could be used as α-glucosidase inhibitor with potential to suppress postprandial hyperglycaemia by reducing the absorption and digestion of carbohydrates.[18] Li et al. (2016) reported *Lactobacillus plantarum* significantly inhibited the activity of the α-glucosidase enzyme.[19] Thus, the α-glucosidase inhibitory activity of *L. casei* and *B. bifidum* was evaluated and compared with acarbose treated positive control group and the result indicated that *L. casei* and *B. bifidum* could be used as potential anti-diabetic probiotic strain for further research.

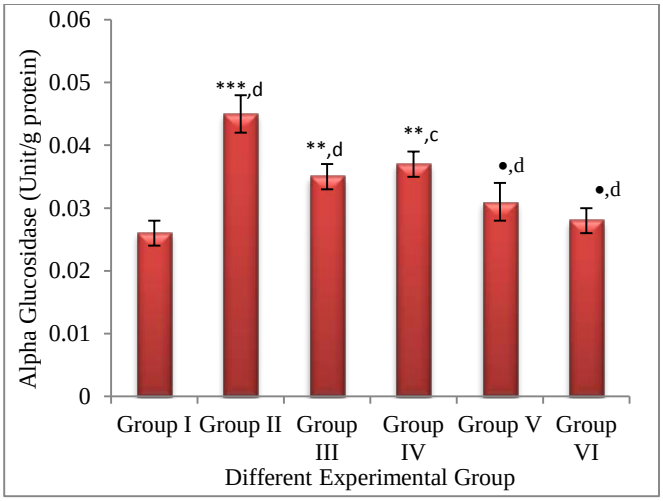

Figure 4. Effect of treatment on alpha glucosidase activity in different experimental groups. Result were presented as mean ± SEM (n=6 animal/group); $p > 0.05$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to healthy control, $^{a}p > 0.05$, $^{b}p < 0.05$, $^{c}p < 0.01$, $^{d}p < 0.001$ compared to diabetic control.

Histological analysis

Histopathological examination of the pancreas was performed (Figure 5) and the finding related to the probiotic protective effect on STZ-induced diabetic rats was obtained through biochemical assays, further confirmed by histological analysis. The effect of acarbose and probiotic treatments on pancreas tissue was histologically examined. The Islets of Langerhans of non-diabetic healthy rats showed normal cellular organization and on the other hand, degenerative and necrotic changes were observed in Islets of Langerhans of the diabetic group. Acarbose and combination treatment of *L. casei* and *B. bifidum* restored such irregularity and improved degenerative and necrotic changes of Islets of Langerhans. The finding indicated that *L. casei* and *B. bifidum* improved degenerated changes of pancreatic Islets in 28 days of the experiment and pancreatic cells recovery was associated with decreasing levels of FBG and PBG. The close link observed between diabetes and pancreatitis that is pancreatitis is associated with oxidative stress, further linked with immunological destruction or glucotoxicity in diabetes.[20] Therefore, to overcome the complications of diabetes, it is necessary to prevent the destruction of pancreatic islets. A previous study reported that the formation of free radicals due to

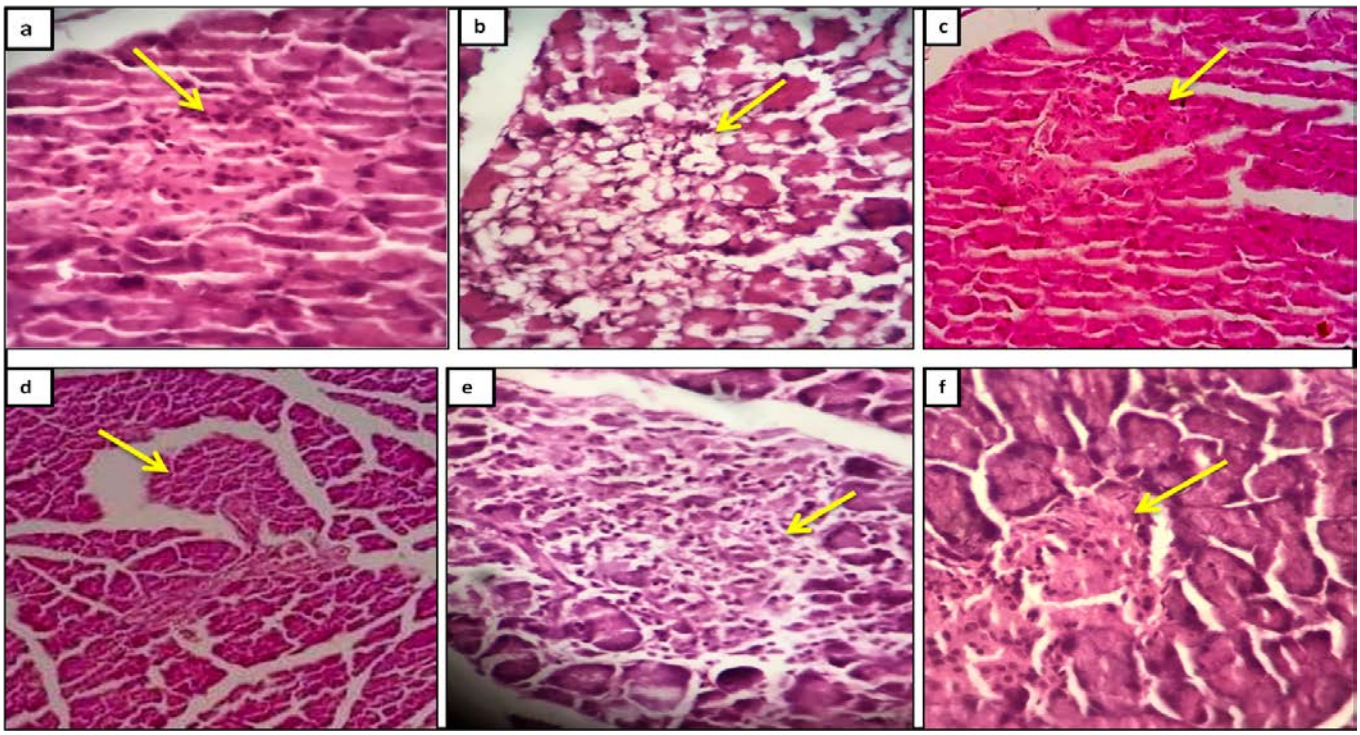

Figure 5. Photomicrograph of stained pancreatic section of different experimental groups (a) Healthy group showing cells of pancreases were present in normal proportion. (b) Diabetic rat section showing degenerative and necrotic changes. (c) *L.casei* treated group-significantly reduce scores of injuries of pancreatic cells. (d) *B. bifidum* treated group showing reduce injury. (e) Combination treated group-treatment showed recovery of islets of pancreatic cells. (f) Acarbose treated normalize the effect of STZ & normal proportion of islets. The arrow represents β Islets of pancreatic tissue. The images were taken at 40x magnification.

auto-oxidation of glucose play a major role in the apoptosis and destruction of pancreatic β-cells[21,22]. Prevention of pancreatic destruction can be achieved by antioxidants. Our previous study demonstrated that oral administration of *L. casei* and *B. bifidum* exerted antioxidant activity and ameliorated oxidative stress.[11]

Chen et al reported that administration of *L. rhamnosus* protected islets of pancreatic cells and reduced β-cells apoptosis.[23] Various studies have reported that the anti-diabetic mechanisms of probiotics might be related to their antioxidant activity. In our 28 days of the experiment, we observed that diabetic animals treated with the combination of *L. casei* and *B. bifidum* recovered necrotic changes in islets of pancreatic cells.

CONCLUSION

The findings of the present study supported the hypothesis that 28 days of treatment with *L. casei* and *B. bifidum* alone and in combination exerts their anti-diabetic effects partly via suppressing α-glucosidase enzyme and also can reduce blood glucose responses after carbohydrate challenges. Administration of *L. casei* and *B. bifidum* alone and in combination during 28 days also protected STZ-induced pancreatic injuries and β-cell function by regeneration of pancreatic islets. The potential hypoglycaemic mechanism of *L. casei* and *B. bifidum* alone and in combination provided a promising approach for the prevention and treatment of diabetes. Thus, these strains could be served as a novel probiotic in the production of probiotic products, food uptake and medication that can reduce blood glucose levels.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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