Anti-hyperglycemic potential of *Lactobacillus* spp. in alloxan-induced Wistar rats

Vandana Bharti[1], Archana Mehta[1], Gajendra Kumar Mourya[2], Laxmi Ahirwal[1], Vivek K Bajpai[3*] and Shruti Shukla[4*]

[1]Department of Botany, Lab of Molecular Biology, Dr. Hari Singh Gour University (Central University), Sagar, MP, India
[2]Department of Biomedical Engineering, School of Technology, North Eastern Hill University, Shillong, Meghalaya, India
[3]Department of Applied Microbiology and Biotechnology, Yeungnam University, Daehak-ro, Gyeongsan-si, Gyeongsangbuk, Republic of Korea
[4]Department of Food Science and Technology, Yeungnam University, Daehak-ro, Gyeongsan-si, Gyeongsangbuk, Republic of Korea

Abstract: This research was aimed to investigate anti-hyperglycemic effects of two *Lactobacillus* spp. on alloxan induced diabetic rats. Alloxan was administered intraperitoneally to induce the diabetic conditions in experimental rats. Animals were treated with oral administration of *Lactobacillus* spp., such as *L. plantarum* and *L. acidophilus* at the dose of 10^8 CFU/ml. As a result, administration of *Lactobacillus* spp. significantly ($P<0.05$) lowered blood glucose levels in diabetic rats by (201-220mg/dl) as compared to diabetic control (265mg/dl). Also, both the *Lactobacillus* spp. were able to reduce body weight of experimental animals as compared to control group, suggesting potent anti-hyperglycemic effect of *Lactobacillus* spp. in terms of their anti-diabetic potential.

Keywords: *Lactobacillus* spp., diabetes, dairy products, body weight.

INTRODUCTION

Diabetes is a chronic disease that occurs when body is unable to produce enough insulin or has complications on its proper use (Steven and Ehrlich, 2012). There is a significant relationship between diabetes and lipid profile abnormalities which can pose a high risk of cardiovascular diseases (Krauss, 2004). Reports of various food agencies have defined the probiotics as live microorganisms which can confer health benefits on host when administered in adequate amounts (Lye *et al*., 2009). Besides, probiotics also exhibit antihypertensive and ant-diabetic properties by modulating lipid profile and insulin resistance (Lye *et al*., 2009).

Although a number of commercial anti-diabetic agents are available in the market to treat diabetic complications (Phung *et al*., 2001), there use has been limited due to severe adversary effects in human being, such as diarrhea, flatulence and bloating (Luna and Feinglos, 2001). Hence, there is a huge interest on using probiotics as health supplements due to their bio-therapeutic potential (Caselli *et al*. 2013). Recent findings have confirmed efficacy of probiotic supplementation on the reduction of lipid and cholesterol levels in animal model (Gomes *et al*. 2014).

Probiotic-based *Lactobacillus* strains have shown enormous potential in the treatment of various chronic diseases, including diabetes and considered safe due to their GRAS nature. A number of lactic acid bacteria are widely distributed in different environments, have been used in the field of food processing for the production of fermented milk in many countries. Recently, probiotic lactic acid bacteria have received a great deal of attention due to their various functional properties (Honda *et al*., 2012).

Probiotic strains *Lactobacillus plantarum* KP894100 and *Lactobacillus acidophilus* KP942831 were previously identified and isolated from dairy products (Bharti *et al*., 2015). In this study, we investigated *in vivo* anti-hyperglycemic effect of *L. plantarum* KP894100 and *L. acidophilus* KP942831 on alloxan induced diabetes in Wistar rats.

MATERIALS AND METHODS

Preparation of bacterial suspensions

Lactobacillus strains, *Lactobacillus plantarum* KP894100 and *Lactobacillus acidophilus* KP942831, used in this study were procured from the Laboratory of Microbiology, Department of Botany, Dr. Hari Singh Gour University. The cultures were incubated in 400ml of de Man Rogosa Sharpe (MRS) broth at 72h. Aliquots were transferred to the sterilized tubes and optical density (OD) at 600 nm was measured until the OD reaches to 0.1 nm. According to McFarland standard, 0.1 nm OD is equivalent to 10^8 cfu/ml of bacterial culture.

Animals and experimentals

Wistar male rats (weighing 200-300g) (Alsayadi *et al*., 2014), were maintained in a well-ventilated room, fed on commercial balanced pellet feed and water *ad libitum* and under standardized conditions. All studies on animals

**Corresponding authors:* e-mails: shruti.shukla15@yahoo.com; vbajpai04@yahoo.com

were approved by the Institutional Animal Ethics Committee (# HSG-Univ. 2015-000065) Department of Pharmaceutical sciences Dr. Hari Singh Gour University, Sagar, Madhya Pradesh, India. The animals were divided into 5 groups after 48 h of the induction of diabetes as followings: Group 1: normal control (treated with sterilized distilled water); Group 2: diabetic control (treated with alloxan at 40 mg/kg body weight); Group 3: positive control (treated with glibenclamide at 50 mg/kg body weight); Group 4: treatment group (treated with *L. plantarum* at 10^8 cfu/ml); and Group 5: treatment group (treated with *L. acidophilus* at 10^8 cfu/ml). Each group consisted 6 animals. Animals were allowed to access for feed and water.

Acute toxicity assay

The rats were kept on fasting, providing only water, after which *L. plantarum* KP894100 and *L. acidophilus* KP942831 were administered orally by gastric tube in different gradual doses (10^6, 10^8, 10^{10}, 10^{12} cfu/ml) then observed for any toxic symptoms and mortality for 72 h.

Preparation of diabetic rats

The acclimatized rats were starved for 24 h with water *ad libitum* and diabetes was induced in rats by intraperitoneally injection of alloxan monohydrate (freshly prepared in normal saline) at a dose of 40 mg/kg b.w. (Saravanan and Pari 2005). After one hour of alloxan administration, animals were given feed *ad libitum*. Blood samples were collected after 24 h of alloxan injection, from the tail vein and blood glucose levels were determined using glucometer (ACCU-Check Active, 3S Healthcare, India). Blood sugar level (250-350 mg/dl) was considered as a diabetic level.

Glucose tolerance test in normal and diabetic rats

Animals of all the groups (diabetic and normal) were fasted overnight (14h) before the glucose tolerance test was done. Thirty minutes following the *Lactobacilli* or glibenclamide treatment, each rat was given an oral glucose load of 3g/kg body weight. Blood samples were withdrawn from the retro-orbital site at 90 min of glucose administration and analyzed for blood glucose levels.

Effect of Lactobacillus strains on body weight

The body weight of each experimental set was measured with an electronic balance (Shenyang Longteng Electronic Co., Ltd., China) on 3rd, 7th, 14th and 21st day.

STATISTICAL ANALYSIS

Data were calculated as mean with standard deviation. One way ANOVA was employed for statistical analysis with a significant level of $P<0.05$.

RESULTS

Acute toxicity study

The bacterial cell suspensions of *L. plantarum* KP894100 and *L. acidophilus* KP942831 were safe up to the highest dose of 10^{12} cfu/ml. The behavior of the animals was clearly observed for the first 8h then at an interval of every 4 h during the next 72h and the bacterial cultures did not display any physical sign of toxicity throughout the experimental period and no mortalities were detected.

Effect of Lactobacillus strains on blood glucose level

Administration of alloxan monohydrate caused a significant elevation in blood glucose levels. The blood glucose levels of the normal control rats, alloxan induced diabetic control rats and diabetic rats treated with glibenclamide and 10^8 cfu/ml *L. plantarum* KP894100 and *L. acidophilus* KP942831 at 90 min after oral administration of glucose (2 g/kg) are shown in fig. 1. *L. acidophilus* KP942831 significantly reduced ($P<0.05$) the blood glucose level at 90 min and remained low over the next 120 min when compared with the diabetic control rats. The serum glucose level of diabetic rats was higher as compared to normal rats and *Lactobacillus* treated rats. The rats treated with both *Lactobacillus* strains showed reduced levels of blood glucose as compared to untreated diabetic rats and significantly different at the level of $P<0.05$. Apart from this, the rats treated with *Lactobacillus* strains exhibited increased level of blood glucose as compare to standard drug (glibenclamide) treated animals.

Effect of Lactobacillus strains on body weight of animals

Results of the effects of both *Lactobacillus* strains at 10^8 cfu/ml/day dose on body weight of alloxan-induced diabetic rats after 21 days of treatment are presented in fig. 2. There was a simultaneous increase in the body weight of normal control rats during the experimental period, while from the 3rd day of experiment, diabetic group showed decrease in body weight. *Lactobacillus* strains treated groups showed ascended body weight pattern. The body weight of the animals treated with *L. acidophilus* (10^8 cfu/ml/day) was ascended and showed similar pattern to that of normal control groups followed by *L. plantarum* (10^8 cfu/ml/day) treated groups. Standard drug glibenclamide showed increasing pattern of body weight continuously during the experimental period.

DISCUSSION

It was clearly observed in our study that both *Lactobacillus* strains revealed significant reduction in body weight of diabetic rats as compared to normal rats. The body weight of *Lactobacillus* strains treated rats was continuously reduced as compared to normal control rats after 7 days. Glibenclamide used as a standard drug was able to boost the body weight with a significant ($P<0.05$) increase by 21% as compared to control group. Interestingly, *L. plantarum* and *L. acidophilus* (10^8 cfu/ml/day) significantly reduced the body weight of diabetic rats (26%) as compared to diabetic control rats.

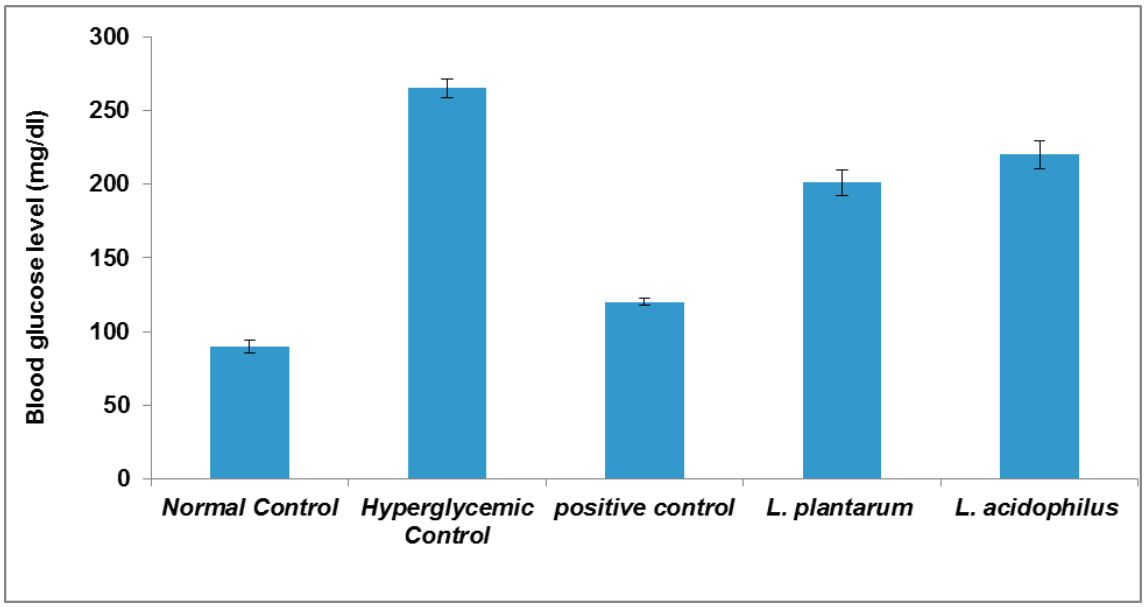

Fig. 1: Effects of *L. plantarum* KP894100 and *L. acidophilus* KP942831 on blood glucose levels of diabetic and control rats. Values are expressed as mean ± SD (n = 6).

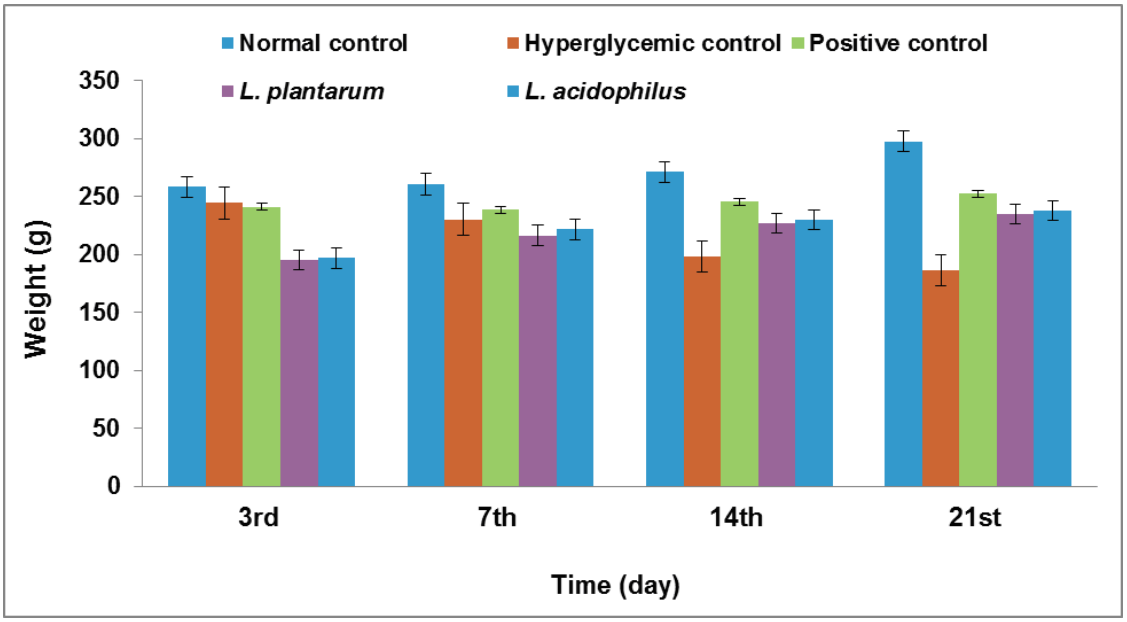

Fig. 2: Effects of *L. plantarum* KP894100 and *L. acidophilus* KP942831 on body weight of alloxan induced diabetic rats after treatment on 3rd, 7th, 14th and 21st day. Values are expressed as mean ± SD (n = 6).

Alloxan as a toxic analogue of glucose has ability to destroy beta-cells in the pancreas when administered to animals resulting in disturbance of insulin production called insulin-dependent diabetes mellitus (Lenzen 2008). Animals with diabetes, obesity, hyperglycemia and cholesterol induced by alloxan exhibit similar disease symptoms as in humans (Chang *et al*. 2006). The excess production of glucose, lipid or decreased utilization of glucose by the pancreatic tissues cause hyperglycemia in diabetes mellitus. Tabuchi *et al*. (2003) reported that *Lactobacillus* GG cells improved glucose tolerance in n-STZ rats and lowered the blood HbA_{1c} level by inhibition of a decrease in insulin secretion. Honda *et al*. (2012) also revealed anti-diabetic potential of viable cells of a lactic acid bacterium *Lactobacillus bulgaricus* which was able to decrease the postprandial blood glucose level in animal model.

Wang *et al*. (2013) determined cardiovascular protectio in abitliy of *L. plantarum* CAI6 and *L. plantarum* SC4 through the regulation of lipid metabolism and by the induction of anti oxidative defense in animal models.

Related to our study, Alsayadi *et al*. (2014) also established an association of probiotic strain of anti-hyperglycemic and anti-hyperlipidemic activities in streptozotocin induced diabetic Wistar rats. The probiotics have a significant role in the treatment or prevention of various chronic diseases, including diabetes (Gomes *et al*., 2014) by lowering the blood glucose levels as also confirmed in this study.

CONCLUSION

Current study confirms that the administration of both *Lactobacillus* strains *Lactobacillus plantarum* KP894100 and *Lactobacillus acidophilus* KP942831 (10^8cfu/ml) was able to reduce the level of blood sugar and weight gain in alloxan-induced diabetic animals, suggesting their anti-hyperglycemic potential. Further strategies are in progress to evaluate the formulation studies of these strains.

ACKNOWLEDGEMENTS

This work was supported by a grant from the University grant commission, New Delhi, India. The authors would like to thank the Department of Pharmaceutical Sciences, Dr. H. S. Gour University, Sagar M.P. India for providing help to do animal experiments and animal housing facility.

REFERENCES

Alsayadi M, Al Jawfi Y, Belarbi M, Soualem-Mami Z, Merzouk H, Sari DC, Sabri F and Ghalim M (2014). Evaluation of anti-hyperglycemic and anti-hyperlipidemic activities of water kefir as probiotic on streptozotocin-induced diabetic Wistar rats. *J. Diabetes Melli.,* **4**: 85-95.

Bharti V, Mehta A, Singh S, Jain N and Ahirwal L (2015). Cytotoxicity of live whole cell, heat killed cell and cell free extract of *Lactobacillus* strain in U087 human glioblastoma cell line and MCF-7 breast cancer cell line. *Int. J. Probio. Prebio*., **10**: 153-158.

Caselli M, Cassol F, Calo G, Holton J, Zuliani G and Gasbarrini A (2013). Actual Concept of 'Probiotics: Is it more functional to science or business? *World J. Gastroenterol.,* **19**: 1527-1540.

Chang S, Hypolite JA, DiSanto ME, Changolkar A, Wein AJ and Chacko S (2006). Increased basal phosphorilation of detrusor smooth muscle myosin in alloxan-induced diabetic rabbit is mediated by upregulation of Rho-kinase β and CPI-17. *Am. J. Physiol. Renal Physiol*., **290**: 650-656.

Gomes AC, Bueno AA, Machado de Souza RG and Mota JF (2014). Gut microbiota, probiotics and diabetes. *Nutr. J.,* **13**: e60.

Honda K, Moto M, Uchida N, He F and Hashizume N (2012). Anti-diabetic effects of lactic acid bacteria in normal and type 2 diabetic mice. *J. Clin. Biochem. Nutr.,* **51**: 96-101.

Krauss R (2004). Lipids and lipoproteins in patients with type 2 diabetes. *Diabetes Care,* **27**: 1496-1504.

Lenzen S (2008). The mechanisms of alloxan- and streptozotocin-induced diabetes. *Diabetologia,* **51**: 216-226.

Luna B and Feinglos MN (2001). Oral agents in the management of type 2 diabetes mellitus. *Am. Fam. Physician.,* **63**: 1747-1756.

Lye HS, Kuan CY, Ewe JA, Fung WY and Liong M. (2009). The improvement of hypertension by probiotics: effects on cholesterol, diabetes, renin and phytoestrogens. *Int. J. Mol. Sci.,* **10**: 3755-3775.

Phung OJ, Baker WL, Tongbram V, Bhardwaj A and Coleman CI (2012). Oral antidiabetic drugs and regression from prediabetes to normoglycemia: A metaanalysis. *Ann. Pharmacother.,* **46**: 469-476.

Saravanan R and Pari L (2005). Antihyperlipidemic and antiperoxidative effect of diasulin, a polyherbal formulation in alloxan induced hyperglycemic rats. *BMC Comp. Alt. Med.,* **5**: e14.

Steven D and Ehrlich N (2012). Solutions Acupuncture: A private practice specializing in complementary and alternative medicine. Diabetes [Online]. Available: http://umm.edu/health/medical/altmed/condition/diabetes [Accessed 13-04 2014].

Tabuchi M, Ozaki M, Tamura A, Yamada N, Ishida T, Hosoda M and Honono A (2003). Antidiabetic effect of *Lactobacillus* GG in streptozotocin-induced diabetic rats. *Biosci. Biotechnol. Biochem.,* **67**: 1421-1424.

Wang LX, Liu K, Gao DW and Hao JK (2013). Protective effects of two *Lactobacillus plantarum* strains in hyperlipidemic mice. *World J. Gastroenterol.* **19**: 3150-3156.