Hypoglycemic and hypolipidemic effects of *Lactobacillus fermentum*, fruit extracts of *Syzygium cumini* and *Momordica charantia* on diabetes induced mice

Sehar Yousaf[1], Abid Hussain[1], Shafiq Ur Rehman[1], Muhammad Shahbaz Aslam[2] and Zaigham Abbas[1]*

[1]Department of Microbiology and Molecular Genetics: University of the Punjab, New campus Lahore, Pakistan
[2]Institute of Biochemistry and Biotechnology, University of the Punjab, Quaid-e-Azam Campus, Lahore, Pakistan

Abstract: A lot of treatment strategies available for diabetes but its complications are still a medical problem around the globe. It demands to find out some alternative therapeutic measures. In order to investigate the anti-diabetic potential of probiotics and natural extracts, this study was designed. Accordingly, a local source of yogurt probiotic strain *Lactobacillus fermentum* was isolated and characterized that showed its probiotic properties. Besides this, natural extracts of plants fruits like java plum (*Syzygium cumini*) and bitter gourd (*M. charantia)* were made. *Lactobacillus fermentum* and the extracts were administered individually as well as in combination to diabetes induced mice. Different parameters like body weight, blood glucose level and lipid profile including total cholesterol, HDL & LDL were analyzed before and after treatment. The results showed that *Lactobacillus fermentum* and natural extracts have hypoglycemic as well hypolipidemic activity against diabetic mice. This study can further investigated to screen potential compounds from these extracts to control the glucose and the lipid levels in diabetic patients.

Keywords: Diabetes, probiotics, *Syzygiumcumini, M. charantia.* natural diabetes therapy. Bitter gourd, Jamun.

INTRODUCTION

Diabetes is a chronic disease that occurs when either an individuals's body is unable to use the insuline or can't produce it. The disease if not treated can lead to other disorders (Steven and Ehrlich, 2012). There is a significant relationshonship between diabetes and lipid profile abnormalities which can pose a high risk of cardiovascular diseases (Krauss, 2004). According to a study in 2011: approximately 366 million masses suffer from diabetes and this number may reach 552 million by 2030 and majority in this list pertain to low and average income countries (Whiting *et al.*, 2011). Despite of the applications of the current tratment strategies against diabetes and its complications, this is still an adressable medical issue. Diabetes imparts two fold risk towards the disorders, such as coronary artery and cardiovascular diseases and hypertension (Collaboration, 2010). FAO/WHO (2002) report defined the probiotics as, "live microorganisms which when administered in adequate amounts confer a health benefit on the host. Besides, other therapeutic properties, probiotics also have antihypertensive and ant-diabetic properties by modulating lipid profile and insulin resistance (Lye *et al.*, 2009).

In India treatment of diabetes with herbs is an old time reality and vegetables are one of the main candidates used for this purpose (Fernandes *et al.*, 2007). Certain plants and herbs have great potential for the therapy of diabetes wherein they directly influence the insulin levels in the blood (Shukia *et al.*, 2000) S*yzygium cumini* is found in various regions of Pakistan and locally it is called Jaman, Jamu or jambolun; it is traditionally used as an agent to cure diabetes (Qureshi *et al.*, 2011;Hussain *et al.*, 2010). *Momordica charantia* in Pakistan is known as "Karela", used as vegetable and its juice is used traditionally for the treatment of diabetese (Ali and Tariq, 2013, Karim *et al.*, 2011, Saeed *et al.*, 2010). *M. charantia* is known to be important for glucose uptake by the cell (Garau *et., al.* 2003). Eugenia jambolana (*Syzygium cumini*) also has hypoglycemic effects (Grover *et al.*, 2000).

This study was conducted to address the use of probiotics and local natural plants extracts as alternative diabetic therapies, which could impart effect on the disorder and its related complications in a natural way with reduced side effects. In future compounds isolated from the extracts of *Syzygium cumini* and *M. charantia* could be screened for hypoglycemic and hypolipidemic potency.

MATERIALS AND METHODS

Isolation and characterization of probiotics

Isolation of probiotics was done from yogurt samples available in local market of Lahore, Pakistan, Preliminary identification by Gram staining, colony morphology; biochemical characteristics and 16srRNA analysis (Tannock, 1999) was also done. The probiotic properties of the isolates were analyzed by following parameters: resistance to acidic pH, bile salt tolerance, growth at different temperatures and different concentrations of

*Corresponding author: e-mail: zaigham.mmg@pu.edu.pk

NaCl, bioactivity, antibiotic resistance, gelatinase activity, arginine hydrolysis (FAO/WHO, 2002; Yavuzdurmaz, 2007).

Preparation of fruit extracts

Java plum (*Syzygium cumini*) and bitter gourd (*M. charantia)* were harvested from Botanical Garden University of the Punjab, Lahore; to prepare their ethanolic and aqueous extracts. While probiotic cell mass was prepared from *Lactobacillus* strain isolated from local yogurt for anti-diabetic study (Ahmed *et al.*, 1998; Grover *et al.*, 2000; Helland *et al.*, 2004).

Anti-diabetic study on streptozotocin induced mice

Experimental animals

All experiments were performed on female mice of 6-8 weeks, with an initial body weight of 21-23 g. Diabetes induction was done by Streptozotocin (150 mg/Kg). Fasting blood glucose level of more than 190 mg/dl was selected as diabetes criteria for the selection of diabetic induced mice.

The probiotic and the extracts were given in the drinking

Experimental design

Seven groups were designed for this study and their details are given in the following table:

Group name	Description
Group 1	Normal healthy mice
Group 2	Diabetes induced mice without treatment
Group 3	Diabetes induced mice treated with M.C
Group 4	Diabetes induced mice treated with E.J
Group 5	Diabetes induced mice treated with L. fermentum
Group 6	Diabetes induced mice treated with L. fermentum + E.J+ M.C
Group 7	Diabetes induced mice treated with Glucophage

M.C: *Momordicacharantia*, E.J: *Eugenia Jambolana (Syzygiumcumini)*

water (distilled, autoclaved). The amounts of administrations were calculated accordingly: Probiotics 0.05% cells (W/V); *Momordica charantia* 200mg/Kg weight of mice, Eugenia jambolana (*Syzygium cumini)* 100 mg/Kg weight of mice and Glucophage 100 mg/Kg weight of mice. They were dissolved in 50 ml of water and administered orally.

Before and after the treatment with Probiotics and natural extracts physical and biochemical analysis for following parameters was done. Body Weight, Blood Glucose estimation was done by Glucometer® (Roche: Germany), Lipid profile (Blood cholesterol, HDL, LDL, Triglycerides) estimation was done by kit method.

RESULTS

Probiotic strains

Probiotics isolates from yogurt were analyzed for their biochemical and probiotic features followed by their identification based on 16srRNA sequence analysis. A competent strain of *Lactobacillus fermentum (Gene Bank Accession* KJ754019*)* was selected for further anti-diabetic studies. For detailed results table 1 and 2.

Assessment of anti-diabetic efficacy of fruit extracts and probiotic bacteria

Body weights of mice

The body weight of all grouped mice before and after each treatment was done and the data desribed in figure 1. There were variations in the body weights of the mice. Firstly, normal control did not show any variation but there was slight reduction in the body weight of untreated diabetic mice and also in diabetic mice treated with E.J. However, the diabetic mice treated with M.C, *L. fermentum*, combined extracts and glucophage showed slight increase in their body weight.

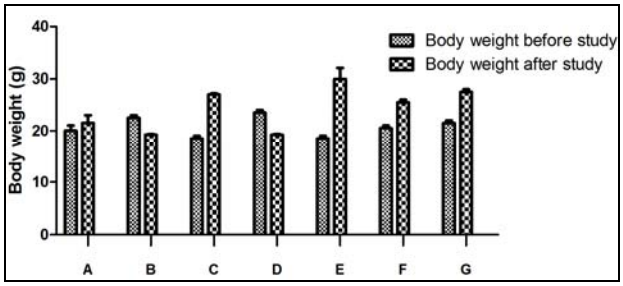

Fig. 1: Body weights of the mice before and after treatment. The body weight of untreated mice decreased but after treatment the weight of the mice in groups C, E, F and G increased which indicates that these drugs helped them to maintain their normal growth. Details of different groups are as follows; (A) Normal healthy mice (B) Diabetes induced mice without treatment (C) Diabetes induced mice treated with M.C (D) Diabetes induced mice treated with E.J (F) Diabetes induced mice treated with *L. fermentum* (G) Diabetes induced mice treated with *L. fermentum* + E.J+ M.C (H) Diabetes induced mice treated with Glucophage.

Estimation of blood glucose level

Blood glucose estimation was done after treatment with different fruit extracts and probiotic bacteria as demostrated in figure 2. Therapeutic agents including M.C, EJ, *L.fermentum*, all combined and glucophage had an appreciable effect in reducing the blood glucose level in diabetes induced mice, however in the untreated diabetes induced mice the blood glucose level was significantly higher.

Table 1: Biochemical feature of the *L.fermentum*strain

Catalase	Glucose	Lactose	Sucrose	Galactose	Ribose
−	++	++	−	++	−

*all biochemical tests were performed in duplicates.
Key: − =No Acid, No Gas; + =Acid Production only ++= Acid and Gas Production

Table 2: Probiotics properties of *Lactobacillus fermentum*

(A)

Acid Tolerance**	Bile salts Tolerance**	Arginine Hydrolysis	Gelatinase activity
Very Good	Very Good	+	+

(B) Growth at Different Temperatures

At 10 ^{0}C	At 40 ^{0}C	At 45 ^{0}C
-	+	-

(C) Growth at Different NaCl concentrations

At 2%NaCl conc.	At 4%NaCl conc.	At 6.5%NaCl conc.
+	+	+

**Lactobacillus fermentum(Gene Bank Accession #* KJ754019*),* *all tests were performed in duplicates, *This strain also had antimicrobial activity against pathogenic Methicillin sensitive strain of *S.aureus* , and was resistant to Streptomycin (15ug), Gentamicin(15ug) and Tetracycline(30ug).
Key; + = Growth; − = No Growth
**Classification criteria included four arbitrary level of acid condition tolerance: excellent if the isolate survived at pH 2/ 0.4% after 24h; very good if the isolate survived at pH 2/ 0.405 after 6h but not after 24h; good if the isolate survived at pH 3/ 0.2% after 24h but not at pH2; poor if the isolate did not survive in any experimental condition. An isolate survived if it demonstrated a surviving percentage equal or more than 50%.

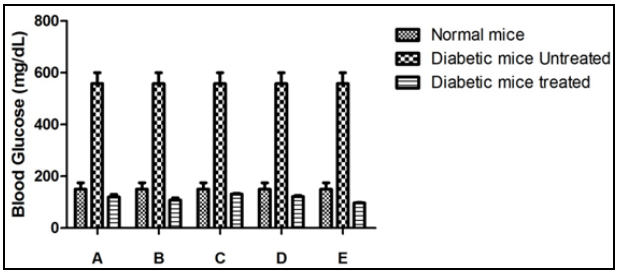

Fig. 2: Effects of alternative therapeutic agents on glucose levels. Mice were administered with different fruit extracts and probiotic bacteria respectively for a period of three weeks. All treatments had hypoglycemic effect as they reduced the levels of glucose in blood. A significant difference in the blood sugar level of treated and untreated diabetic mice was observed. Details of the different treatment groups are as follows; (A) *Monomordicacharantie* (B) *Eugenia Jambolana*(C) *L. Fermentum* (D) *MC+EJ+L. Fermentum* (E) *Glucophage.*

Effects of anti-diabetic treatments on Lipid profile of the mice

Diabetic untreated mice showed significant increase in serum cholesterol levels, while diabetes induced mice treated with M.C and *L. fermentum* had notable positive effect on cholesterol level reduction (fig. 3).

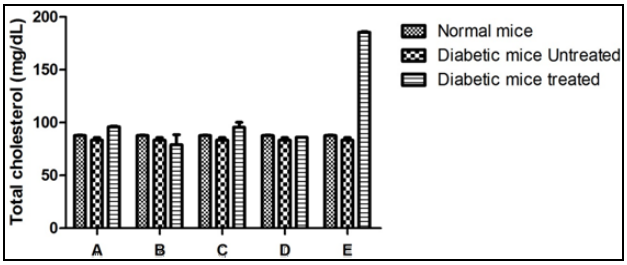

Fig. 3: Effect of treatments on serum total cholesterol. The blood was collected and allowed to clot to get serum and then the serum total cholesterol was measured. The significant results were shown with *Momordicacharantia* and *L. fermentum* treatments. Details of the different treatment groups are as follows; (A) *Monomordicacharantie* (B) *Eugenia Jambolana*(C) *L. Fermentum* (D) *MC+EJ+L. Fermentum* (E) Glucophage.

The treatment with MC and combined mixture was shown to lower the triglycerides level in diabetic mice while other alternative treatments also could not help to reduce triglyceride levels. Detailed results are presented in figure 4.

HDL is thought to be good cholesterol, its elevated level is considered to have a good impact. In this study,

treatment with E.J and *L. fermentum* showed slight increase in the level of HDL while its level remained constant in Glucophage treated diabetic mice (figure 5).

On the other side this study revealed that untreated diabetic mice showed slight increased level of LDL, while diabetic mice treated with MC, EJ, *L. fermentum* showed reduction in LDL level, however all combined mixture did not show positive effect on LDL level as shown in figure 6.

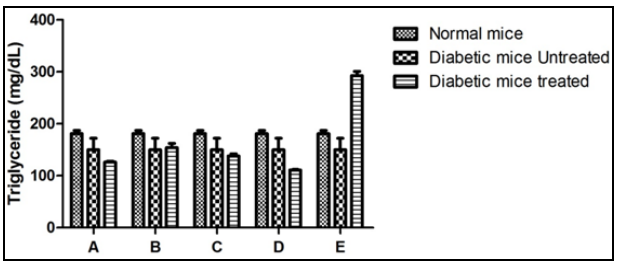

Fig. 4: Effects of treatment on diabetic mice triglycerides levels.All alternative treatments did not allow the triglycerides level to elevate from the normal range. Details of the different treatment groups are as follows; (A) *Monomordicacharantie* (B) *Eugenia Jambolana*(C) *L. Fermentum* (D) *MC+EJ+L. Fermentum*(E)Glucophage.

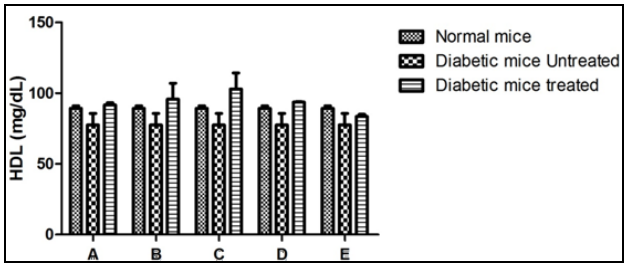

Fig. 5: Effect of treatment on HDL level: HDL is considered as the good cholesterol, our study showed that treated mice showed slight increased level of HDL as compared to untreated mice. Details of the different treatment groups are as follows; (A) *Monomordicacharantie* (B) *Eugenia Jambolana* (C) *L. Fermentum* (D) *MC+EJ+L. Fermentum* (E) Glucophage.

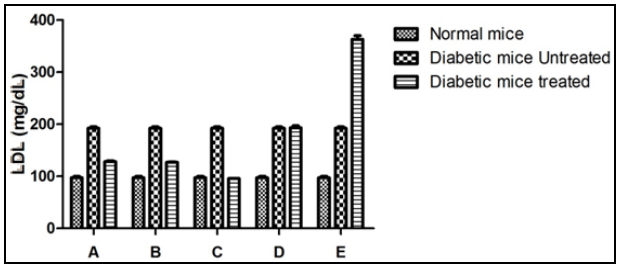

Fig. 6: Treatment effect on LDL level: study showed that *L. ferementum* comparatively has good effect in lowering LDL level in diabetic mice. Details of the different treatment groups are as follows; (A) *Monomordicacharantie* (B) *Eugenia Jambolana* (C) *L. Fermentum* (D) *MC+EJ+L. Fermentum* (E) *Glucophage.*

DISCUSSION

Diabetes being one of the leading medical issues of current times, demands to find out more alternative therapeutic measures along with conventional treatments, in order to effectively overcome this problem. Conventional treatments are effective to control the sugar levels but are less effective enough to overcome lipid profile abnormalities which further lead to other complications such as cardiovascular diseases. In this study we assessed natural fruit extracts and probiotic bacteria as an alternative therapeutic agent to affect body weight, glucose level and lipid profile of the diabetic mice.

After treatment normal control did not show any variation in their body weights however there was slight reduction in the body weight of untreated diabetic mice, which could be due to the cells are in starvation and utilizing more and more body store to meet the needs of body. Blood sugar levels are raised in diabectic individuals but their cells cannot uptake the glucose due to absence of insulin and get starved (Kasetti *et al.*, 2010). The diabetic mice treated with M.C, *L. fermentum*, combined extracts and glucophage showed slight increase in their body weight and this might be due to the fact that M.C has positive effect on the mice metabolism and reduces the complications of diabetes (Chen *et., al.* 2003). The use of E.J as treatment of diabetes reduced the body weight of mice which is parallel to an earlier reported study in which E.J reduced both body weight and blood sugar level in diabetic mice (Grover *et al.*, 2000).

Other than body weight, blood glucose estimation is the basic criterion to evaluate the effectiveness of the anti-diabetic therapy. In this study therapeutic agents including M.C, EJ, *L.fermentum*, all combined and glucophage have an appreciable effect in reducing the blood glucose level in diabetes induced mice while in the untreated diabetes induced mice the blood glucose level was higher. According to Lo *et al.* (2013), novel peptides of *Momordica charantia* are involved in maintaining the blood glucose level, mainly trypsin inhibitor (TI), a novel peptide which regulates the insulin signaling pathway. *Syzygium cumini* has anti α-glucosidase activity in rats (Shinde *et al.*, 2008). *L. fermentum* isolated from yogurt was able to lower fasting blood glucose level and has anti-oxidative ability, so it may be helpful in the treatment of diabetes (Ejtahed *et al.*, 2012).

Natural fruit extracts and probiotic bacteria were found effective to control not only blood glucose level but also lipid profile of the diabetic mice. Treatment with M.C and *L. fermentum* notably reduced cholesterol level in diabetes induced mice however, glucophage (a conventional anti-

dabetic medicine) did not affect positively on the level of cholesterol. Similarly, treatment with fruit exatrcts and *L. fermentum* affect positively on the level of LDL, HDL and triglycerides of diabetic mice. It is in accordance with previous studies which showed that MC, EJ, *L.fermentum* have hypoglycemic as well hypolipidemic activity and could be helpful in the management of diabetes mellitus and associated complications (Jayasooriya *et al.*, 2000; Kasetti *et al.*, 2010; Pereira *et al.*, 2003).

CONCLUSION

Current study was designed to analyze the local natural resources which may help in the therapy of diabetes and its manifestations like hyperlipidemia which could cause cardiovascular disorders. Present study showed that *M. charantia, Syzygium cumini* and, *L. fermentum* have positive effect on lipid profile as well as on lowering blood glucose level in diabetes induced mice. Further studies will help to screen functional components of the extracts and their exact mechanism of glucose and lipid control.

ACKNOWLEDGEMENT

This research work was funded by University of the Punjab, Lahore, Pakistan.

REFERENCES

Ahmed I, Adeghate E, Sharma A, Pallot D and Singh J (1998). Effects of *Momordica charantia* fruit juice on islet morphology in the pancreas of the streptozotocin-diabetic rat. *Diabetes Res. Clin. Pract.,* **40**: 145-151.

Ali S and Tariq A (2013). Analysis of secondary metabolites in callus cultures of *Momordica charantia* cv. Jaunpuri. *Biological Society of Pakistan,* **59**: 23-32.

Chen Q, Chan LL and Li ET (2003). Bitter melon (Momordica charantia) reduces adiposity, lowers serum insulin and normalizes glucose tolerance in rats fed a high fat diet. *The Journal of Nutrition*, **133**: 1088-1093.

Collaboration ERF (2010). Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies. *The Lancet,* **375**: 2215-2222.

Ejtahed HS, Mohtadi-Nia J, Homayouni-Rad A, Niafar M, Asghari-Jafarabadi M and Mofid V (2012). Probiotic yogurt improves antioxidant status in type 2 diabetic patients. *Nutrition,* **28**: 539-543.

FAO/WHO (2002). Guidelines for the evaluation of probiotics in food. London, Ontario, Canada FAO/WHO Working Group Report. 1-11.

Fernandes NP, Lagishetty CV, Panda VS and Naik SR (2007). An experimental evaluation of the antidiabetic and antilipidemic properties of a standardized *Momordica charantia* fruit extract. *BMC Complement. Altern. Med.,* **7**: 29.

Garau C, Singh J and Cummings E (2003). Beneficial effect and mechanism of action of Momordica charantia in the treatment of diabetes mellitus: A mini review. *Int. J. Diabetes*, **11**: 46-55.

Grover J, Vats V and Rathi S (2000). Anti-hyperglycemic effect of Eugenia jambolana and *Tinospora cordifolia* in experimental diabetes and their effects on key metabolic enzymes involved in carbohydrate metabolism. *J. Ethnopharmacol.,* **73**: 461-470.

Helland MH, Wicklund T and Narvhus JA (2004). Growth and metabolism of selected strains of probiotic bacteria in milk-and water-based cereal puddings. *Int. Dairy J.,* **14**: 957-965.

Hussain K, Nisar MF, Majeed A, Nawaz K and Bhatti KH (2010). Ethnomedicinal survey for important plants of Jalalpur Jattan, District Gujrat, Punjab, Pakistan. *Ethnobotanical Leaflets*, **11**: 111-117.

Jayasooriya AP, Sakono M, Yukizaki C, Kawano M, Yamamoto K and Fukuda N (2000). Effects of *Momordica charantia* powder on serum glucose levels and various lipid parameters in rats fed with cholesterol-free and cholesterol-enriched diets. *J. Ethnopharmacol.,* **72**: 331-6.

Karim A, Nouman M, Munir S and Sattar S (2011). Pharmacology and Phytochemistry of Pakistani herbs and herbal drugs used for treatment of diabetes. *International Journal of Pharmacology,* **7**: 419-439.

Kasetti RB, Rajasekhar MD, Kondeti VK, Fatima SS, Kumar EGT, Swapna S, Ramesh B and Rao CA (2010). Antihyperglycemic and antihyperlipidemic activities of methanol: water (4: 1) fraction isolated from aqueous extract of *Syzygium alternifolium* seeds in streptozotocin induced diabetic rats. *Food Chem. Toxicol.,* **48**: 1078-1084.

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

Lo HY, Ho TY, Lin C, Li CC and Hsiang CY (2013). *Momordica charantia* and its novel polypeptide regulate glucose homeostasis in mice via binding to insulin receptor. *J. Agric. Food Chem.,* **61**: 2461-2468.

Lye HS, Kuan CY, Ewe JA, Fung WY and Liong MT (2009). The improvement of hypertension by probiotics: effects on cholesterol, diabetes, renin, and phytoestrogens. *International Journal of Molecular Sciences,* **10**: 3755-3775.

Pereira DI, Mccartney AL and Gibson GR (2003). An *in vitro* study of the probiotic potential of a bile-salt-hydrolyzing *Lactobacillus fermentum* strain, and determination of its cholesterol-lowering properties. *Appl Environ Microbiol,* **69**: 4743-4752.

Qureshi R, Maqsood M, Arshad M and Chaudhry AK (2011). Ethnomedicinal uses of plants by the people of Kadhi Areas of Khushab, Punjab, Pakistan. *Pak. J. Bot.,* **43**: 121-133.

Saeed M, Shahzadi I, Ahmad I, Ahmad R, Shahzad K, Ashraf M and Nisa V (2010). Nutritional analysis and antioxidant activity of bitter gourd (*Momordica charantia*) from Pakistan. *Pharmacologyonline,* **1**: 252-260.

Shinde J, Taldone T, Barletta M, Kunaparaju N, Hu B, Kumar S, Placido J and Zito SW (2008). Alpha-glucosidase inhibitory activity of *Syzygium cumini* (Linn.) Skeels seed kernel in vitro and in Goto-Kakizaki (GK) rats. *Carbohydr. Res.,* **343**: 1278-1281.

Shukia R, Sharma, S, Puri D, Prabhu, K and Murthy P (2000). Medicinal plants for treatment of diabetes mellitus. *Indian J. Clin. Biochem.,* **15**: 169-177.

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].

Tannock GW (1999). Identification of lactobacilli and bifidobacteria. *Curr. Issues Mol. Biol.*, **1**: 53-64.

Whiting DR, Guariguata L, Weil C and Shaw J (2011). IDF diabetes atlas: Global estimates of the prevalence of diabetes for 2011 and 2030. *Diabetes Res. Clin. Pract.,* **94**: 311-321.

Yavuzdurmaz H (2007). Isolation, characterization, determination of probiotic properties of lactic acid bacteria from human milk. M. Sc. Thesis, The graduate School of Engineering and Sciences of Izmir Institute of Technology, pp.1-69.