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

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**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, *Syzygium cumini*, *M. charantia*. natural diabetes therapy. Bitter gourd, Jamun.

**INTRODUCTION**

Diabetes is a chronic disease that occurs when either an individual’s body is unable to use the insulin or can’t produce it. The disease if not treated can lead to other disorders (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). 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 treatment strategies against diabetes and its complications, this is still an adrssable 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 anti-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) *Syzygium 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 diabetes (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 16s rRNA 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...
Hypoglycemic and hypolipidemic effects of Lactobacillus fermentum, fruit extracts of Syzygium cumini


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 water (distilled, autoclaved). The amounts of administrations were calculated accordingly: Probiotics 0.05% cells (W/V); M. 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 described 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 L. fermentum (E) Diabetes induced mice treated with E.J (F) Diabetes induced mice treated with L. fermentum + E.J+ M.C (G) 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 demonstrated 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.
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) *Monomordica charantia* (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).

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

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<tr>
<th>Catalase</th>
<th>Glucose</th>
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<th>Galactose</th>
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*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

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(B) Growth at Different Temperatures

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*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%.

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Key; + = Growth; − = No Growth

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**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) *Monomordica charantia* (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,
Hypoglycemic and hypolipidemic effects of Lactobacillus fermentum, fruit extracts of Syzygium cumini

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.

**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 antioxidative 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-
studies will help to screen functional components of the blood glucose level in diabetes induced mice. Further positive effect on lipid profile as well as on lowering extracts and their exact mechanism of glucose and lipid control.

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

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REFERENCES


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