ORIGINAL ARTICLE

COMPARISON OF EFFECTS OF PROBIOTICS AND SITAGLIPTIN ON SERUM GLUCOSE IN DIABETIC RATS

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Background: Diabetes mellitus has become a major disease having catastrophic effects on health and economy globally. This study was conducted to determine the role of Probiotics in comparison to Sitagliptin in decreasing serum glucose levels in diabetic rats. **Methods:** Fifty male rats were divided into two groups: Normal control group-A (n=10) and Experimental group-B (n=40). Group-B was administered Streptozotocin to induce diabetes, which was confirmed after one week by measuring Fasting Blood Glucose (FBG) (>7 mmol/L were considered diabetic). Then group B was subdivided into BI (Diabetic Control), BII (Sitagliptin treated), BIII (Probiotics treated) and BIV (Sitagliptin plus Probiotics treated) with 10 rats each. Baseline serum glucose was measured at day zero. Next sampling was done on day 40, after administering Probiotics (250 mg/Kg) and Sitagliptin (10 mg/Kg). Terminal sampling was done on day 60. Statistical analysis was done on SPSS-21. Comparisons between the groups were analysed using one-way ANOVA (post-hoc Tuckey test), and $p<0.05$ was considered statistically significant. **Results:** Rats in Probiotic treated group-BIII had significant reduction in fasting blood glucose levels with efficacy comparable to Sitagliptin in group-BII ($p<0.05$). Synergistic effect of Sitagliptin plus Probiotics in group-BIV was greater ($p<0.05$) than their individual effects in groups-BII and BIII. **Conclusion:** Probiotics significantly decrease fasting blood glucose in diabetic rats with efficacy comparable to Sitagliptin. Synergistic effect of Sitagliptin plus Probiotics is greater than their individual effects.

Keywords: Fasting blood glucose, diabetes mellitus, Probiotics

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INTRODUCTION

Diabetes mellitus (DM) has become one of the major diseases in the world with its catastrophic effects on health and economy around the globe.[1,2] The burden of this disease has increased four times for the past three decades due to increase in obesity, sedentary lifestyle, consumption of high calorie food and ageing of population.[3] In the present era, there is rising trend of developing diabetes mellitus in childhood and adolescence.[4] Chronically disrupted metabolism in diabetic patients increases the risk for vascular complications and frequent hospitalizations.[3]

World health organization (WHO) reports around 171 million people globally are suffering from diabetes, with 82 million diabetic cases in the South East Asia.[5] In 2030, the diabetes prevalence will be 67%.[6] The Second National Diabetes Survey of Pakistan (2nd NDSP) 2016–17 reports, the prevalence of diabetes in Pakistan to be 26.3%, out of which 19.2% were old cases and 7.1% were newly diagnosed cases of diabetes. Diabetes prevalence in urban and rural areas has been reported as 28.3% and 25.3% respectively.[7]

Streptozotocin is used to induce diabetes in rat model, because it causes partial destruction of the beta cells. An inflammatory response is activated leading to auxiliary loss of beta cell activity and results in developing DM.[7] Oral dipeptidyl peptidase-IV (DPP-IV) inhibitors have emerged as newer hypoglycaemic agents which enhances the biological effects of incretin hormones by inhibiting their breakdown by dipeptidyl peptidase-IV enzyme.[8] Sitagliptin is the first oral DPP-IV inhibitor discovered recently.[9] Innumerable studies have established that Probiotics hold wonderful anti-diabetic activity and restore the action of pancreatic β-cells. Probiotics are safe, easily available, economical, eco-friendly, and provide a permanent cure of the disease.[10]

The treatment protocol of diabetes include both non-pharmacological (diet, exercise) and pharmacological measures (insulin and oral hypoglycaemic agents).[11] It is proposed that the modern approach to treatment of diabetes needs to be based upon prior identification of the cause and then treating the underlying biological abnormalities.[12] Therefore, the treatment approach towards diabetes should be to normalize or at least reduce the underlying physiological derangements in diabetes.

In a country like Pakistan, where a large population is living a life below the line of poverty, proposing expensive treatment strategies is not justified. Besides the economic constrains, the adverse effects of these medicines, prompt us to look for a safe alternative.[13] Use of Probiotics having good metabolic properties has the potential to be a better alternate

therapy for treating diabetes mellitus and its complications.[14] Limited data is available in Pakistan to highlight the uses of locally available commercial preparations of Probiotics. No study has been conducted so far for comparative analysis of Probiotics and Sitagliptin in Pakistan. This study will be a useful tool to bridge this gap in the field of diabetes management with commercial Probiotics preparations available for use by the society.

METHODOLOGY

This quasi experimental study was carried out at Pharmacology Laboratory, Multidisciplinary Research Laboratory, Islamic International Medical College, and National Institute of Health, Islamabad. Formal approval by the Ethics Review Committee of Islamic International Medical College, Riphah International University, was obtained for this work. The study was conducted from September 2020 to August 2021. A total of 50 male rats weighing 200–250 grams, without any physical abnormality and having normal baseline parameters were procured from NIH animal house.

All the rats were accommodated in standard cages at the Animal House of NIH, Islamabad. The rats had free access to tap water. Animal house atmosphere was maintained at 20±2 °C with relative humidity of 50–70% and a light and dark cycle of 12 hours each. After acclimatization for 1 week, the rats were divided into two main groups; 10 rats were allocated to group A, and 10 rats each to group BI, BII, BIII and BIV. Group A was labelled as Normal Control whereas the group B (Experimental group) was administered single intra-peritoneal injection of streptozotocin dissolved in sodium citrate buffer at dose of 40 mg/Kg. Diabetes confirmation was done after one week in experimental group by measuring fasting blood glucose (FBG) and comparing it with Group A. Rats with fasting blood glucose >7 mmol/L were considered as diabetic.[1] The experimental group B was subdivided into four groups as BI, BII, BIII, and BIV.

Group A (n=10) had normal healthy rats with no intervention, having free access to food and water. Group B (n=10 in each group) had Streptozotocin induced diabetic rats, and were further subdivided into 4 groups. Group BI was diseased control group which did not receive any treatment and received normal saline with normal standard diet only. Group BII was diabetic rats which received Sitagliptin at the dose=10 mg/Kg/day.[15] Group BIII had diabetic rats receiving Probiotics at the dose=250 mg/rat/day[16] and Group BIV had Diabetic rats which received Sitagliptin (10 mg/Kg/day)[15] and Probiotics (250 mg/rat/day).[16]

A mid-cycle sampling was done after 40 days to see the progress of drugs on serum glucose levels. At day 60, final sampling of the experiment was done by cardiac puncture in all groups to see effect on serum glucose levels.[17]

Statistical analysis was done on SPSS-21. Results were documented as mean differences. Comparisons of quantitative parameters among the groups were analysed using one way ANOVA (post hoc Tuckey test), and $p<0.05$ was considered as significant.

RESULTS

Table-1 shows the comparison of Mean difference of all the groups. The results of group BII, BIII and BIV were compared to group BI, the diabetic control group. In the below mentioned table, the significant results are verified which are compared with the disease control group. In Probiotics plus Sitagliptin treated group BIV, there was a substantial drop in hyperglycaemia as compared to group BI, the diabetic control group. The results also imply that the synergistic effect of Sitagliptin plus Probiotics as a treatment tool for diabetes is greater than the effect of each drug individually in groups BII and BIII respectively.

Table-1: Comparison of mean difference of serum fasting blood glucose (mg/dl) in all groups on day 60

Groups	Mean difference	*p*
A *vs* B1	416.00	0.000*
A *vs* B2	10.10	0.04*
A *vs* B3	13.60	0.007*
A *vs* B4	6.00	0.21
B1 *vs* B2	405.90	0.000*
B1 *vs* B3	402.40	0.000*
B1 *vs* B4	410.00	0.000*
B2 *vs* B3	3.50	0.47
B2 *vs* B4	4.10	0.39
B3 *vs* B4	7.60	0.12

*Significant

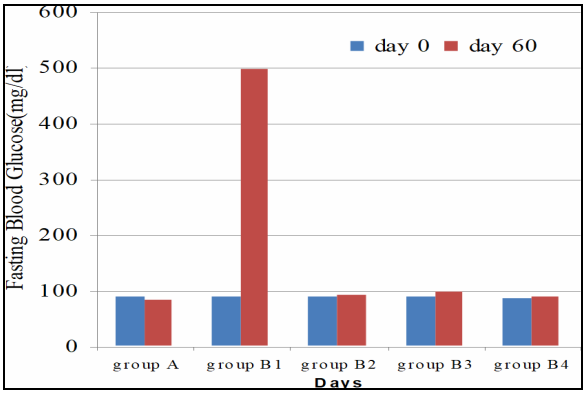

Figure-1: Means of fasting blood glucose levels of all groups on Day 0 and Day 60 (n=50)

DISCUSSION

In present study, the anti-diabetic effect of Probiotics in comparison to sitagliptin is observed. Results of the present study confirm that hyperglycaemia induced by streptozotocin, is ameliorated by Probiotics with efficacy comparable to sitagliptin.

Various studies have reported that Probiotics have significant anti-diabetic effects.[18] Our study, revealed that the group treated with Probiotics had decreased FBG by reversing hyperglycaemia which is in agreement with Sohag MSU *et al*[19] who studied that administration of Probiotics decreased FBG in diabetic rats. Aggarwal J *et al*[20] studied the use of Probiotics and their anti-hyperlipidaemic action in streptozotocin-induced diabetic mice. A study by Yadav R *et al*[21] revealed that Probiotics supplementation improved metabolic profile in the diabetic subjects and caused reduction in their FBG, HbA1c and triglycerides.

No work has been done in Pakistan to compare the anti-diabetic effects of Probiotics with the anti-diabetic drug Sitagliptin. We chose Sitagliptin as it is a newer anti-diabetic drug with better qualities as compared to previous drugs.[22] Also the probiotic strain Lactobacillus has DPP-4 inhibitory activity like Sitagliptin, which was studied by Yan F *et al*[23]. This implies that their combined use will lead to synergistic effects in managing diabetes.

Most of the studies conducted for effects of Probiotics on diabetes were either on cultures of Probiotics[1] or Probiotics fermented products, e.g., probiotic fermented milk[24] or yogurt[25]. We used commercial preparation of Probiotic because of its easy availability. Such preparations are economical and for the sake of patient compliance they are easy to administer, i.e., via oral route. This was in accordance to a study conducted by Campos LF *et al*[16] who used commercial preparation of Probiotics with the name Protégé. The Protégé commercial probiotic preparation contained Lactobacillus and Bifidobacterium strains of Probiotics. Hiflora™, a commercial and locally available, probiotic preparation in Pakistan, that we used in our study also contained Lactobacillus and Bifidobacterium strains of Probiotics.

CONCLUSION

The synergistic effect of Sitagliptin plus Probiotics in lowering serum glucose in diabetic rats is greater than the effect of individual drugs. Probiotics significantly lower fasting blood glucose level in diabetic rats' model with efficacy comparable to Sitagliptin. Probiotics can be used as an adjunct in treatment for diabetes mellitus.

RECOMMENDATIONS

Comparative effects of different doses and roots of administration, besides other potential beneficial effects of Probiotics may be further investigated.

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FMB: Concept, acquisition, Analysis and manuscript writing
AW: Concept, analysis and direction
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RI: Revision of project and review of content
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