EFFECT OF BIFIDOBACTERIUM AND LACTOBACILLUS ACIDOPHILUS IN DIABETIC RATS

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*Abeer El Sayed El Khamisy*

**Abstract**

Insulin and oral hypoglycemic agent are the main ways to treat diabetes mellitus and are effective in controlling hyperglycemia, but these kinds of drugs also have prominent side effects. Bifidobacterium and Lactobacillus acidophilus have attracted a lot of attention for their potential probiotic effects in human health. So that the aim of this study is to investigate the hypoglycemic effects of Bifidobacterium ($10^8$ cfu/day) and Lactobacillus acidophilus ($10^8$ cfu/day) alone and in combinations in diabetic rats and their effects on liver and kidney functions.

A total of 30 male rats weighing ($160 \pm 5$ g) were randomly assigned to five dietary treatment groups as follow: the first group (control negative) fed on basal diet only. Groups from 2 to 5 are diabetic, the second group (control positive) fed on basal diet only. The third group fed on basal diet and given orally Lactobacillus acidophilus. The fourth group fed on basal diet and given orally Bifidobacterium. The fifth group fed on basal diet and given orally a combination of (Lactobacillus acidophilus and Bifidobacterium ). At the end of the experimental period (6 weeks) blood was collected then glucose, insulin, lipid profile and liver and kidney functions were determined in the serum. The results indicated that, supplementation with Bifidobacterium and Lactobacillus acidophilus alone and in combination significantly decreased the mean value of serum glucose, total cholesterol, triglycerides, LDL-C and VLDL-C and significantly increased HDL-C and insulin secretion as compared to control groups. Also, Bifidobacterium and Lactobacillus acidophilus alone and in combination significantly improved liver and kidney function of diabetic rats. The use of Bifidobacterium and Lactobacillus bacteria was more effective than the use of one.

**Key words:** Bifidobacterium - Lactobacillus- glucose- insulin – lipid profile
INTRODUCTION

Introduction

Diabetes mellitus is a major endocrine disorder, affecting nearly 10% of the population all over the world. In spite of the introduction of hypoglycemic agents, diabetes and the related complications continue to be a major medical problem (Nammi et al., 2003). Diabetes is associated with the generation of reactive oxygen species (ROS), causing oxidative damage particularly to various tissues (Mohamed et al., 1999). Hyperglycemia can cause oxidative stress, which, in turn, may result in cellular tissue damage. The harmful influence of diabetes on metabolism of tissues and organs is well known. Likewise, uncontrolled hyperglycemia can lead to disturbances in the structure and functions of organs (Gupta et al., 2004).

Examples of probiotics are Lactobacillus rhamnosus GG, Lactobacillus acidophilus, and Bifidobacterium bifidum (Perdigon et al., 1995). Probiotics are live microbial organisms that are administrated as supplements or in foods to benefit the host. Probiotics occur naturally in fermented food products such as yoghurt. Numerous health benefits have been attributed to probiotics, including effects on gastrointestinal tract function and diseases, immune function, hyperlipidemia, hypertension, and allergic conditions (Nichols, 2007).

Hariom et al., (2007) investigated the effect of low-fat (2.5%) dahi containing probiotic Lactobacillus acidophilus and Lactobacillus casei on progression of high fructose-induced type 2 diabetes in rats. The probiotic dahi-supplemented diet significantly delayed the onset of glucose intolerance, hyperglycemia, hyperinsulinemia, dyslipidemia, and oxidative stress in high fructose-induced diabetic rats, indicating a lower risk of diabetes and its complications.

Al-Salami et al., (2008) investigate the influence of probiotics on gliclazide pharmacokinetics and the effect of both probiotics and gliclazide on blood glucose levels in healthy and diabetic rats. Diabetic rats were gavaged with probiotics (75 mg/kg) for three days after which a gliclazide suspension (20 mg/kg) was administered by gavage to all groups. The results indicated that, probiotic treatment of diabetic rats increases gliclazide bioavailability and lowers blood glucose levels by insulin-independent mechanisms, suggesting
that the administration of probiotics may be beneficial as adjunct therapy in the treatment of diabetes.

Bifidobacterium and Lactobacillus acidophilus have attracted a lot of attention for their potential probiotic effects in human health. B. longum ATCC 15708, an infant intestine isolate, and L. acidophilus ATCC 4356, a human isolate, are used as dietary adjuncts in various cultured dairy products. Therefore, the objective of this study was to investigate the hypoglycemic effects of Bifidobacterium and Lactobacillus acidophilus alone and in combinations in diabetic rats and their effects on liver and kidney functions.

Materials and methods:

Materials

Adult male albino rats of Sprague Dawley strain were obtained from Helwan Farm, Ministry of Health and Population, Cairo, Egypt. Chemicals: Casein, cellulose, vitamins, minerals and streptozotocin were obtained from the General Company for Commerce and Chemicals. Kits were purchased from Gama Trade Company for chemicals, Cairo, Egypt. Bacterial cell haves (3.2.1) was obtained from Misr food additives company. MRS broth haves (3.2.2) was obtained from Oxford.

Methods:

Preparation of bacterial suspension:

1. Five gm from each of freeze dried lactobacillus acidophilus and bifidobacteria lactis (3.2.1) were mixed separately with 45 ml of sterile MRS broth (3.2.2) in sterile Erlenmyr's flask and incubated anaerobically at 37 °C for 48-72 hrs.

2. Counting the colony forming unit per ml of the incubated broth was performed for both species using MRS agar (3.2.2) which was incubated at 37 °C for 48-27 hrs.

3. Dilution was made for both microorganisms in 1 liter of brain heart infusion broth to give final concentration of $10^8$ cfu/ml.

4. Prepared bacterial suspension were stored at 4 °C till the beginning of the experiment (Heon, et al., 2007).

Induction of diabetic rats:

Rats were injected with streptozotocin (60 mg/kg body weight) to induce hyperglycemia according to the method of (Adeghate et al., 2001) fresh solution of streptozotocin was prepared in phosphate citrate buffer PH= 4.6...
suitable for dissolving it just before injection. After 120 hours of the injection a blood sample was taken from each rat for the determination of glucose to ensure the occurrence of diabetes.

**Diets and Experimental Design :**

Animal diets were formulated based on AIN 1995. The basal diet (g/kg diet) consisted of 140 gm casein (> 80 % protein) , 100 gm sucrose , 50 gm corn oil , 50 gm cellulose, 35 gm mineral mixture, 10 gm vitamin mixture, 1.8 gm L-cystine, 2.5 gm choline bitartrate and the remainder is corn starch. Diets were formulated according to (Reeves , et al., 1993). A total of 30 male Sprague-Dawley rats initially weighing (160 ±5 g), were housed in individual stainless steel cages. Rats were randomly assigned to five dietary treatment groups of six rats each. After the adaptation period, rats were divided into five groups, (each with 6 rats) as follow: The first group (control negative) fed on basal diet only. Groups from 2 to 5 are diabetic, The second group (control positive) fed on basal diet only. The third group fed on basal diet and given orally Lactobacillus acidophilus. The fourth group fed on basal diet and given orally Bifidobacterium. The fifth group fed on basal diet and given orally a combination of (Lactobacillus acidophilus and Bifidobacterium ). At the end of the experimental period (6 weeks) rats fasted over night before sacrificing, blood were collected then centrifuged. Serum were separated and stored at - 20\(^{0}\) C until analysis.

**Chemical Analysis:**

Serum glucose was determined according to (Tinder, 1969). Serum total cholesterol was determined according to (Flegg,1973). Serum triglyceride was estimated by (Buccolo and David, 1973) . HDL-C was estimated by (Kostner, 1977). LDL- C was estimated by to Friedewalde, et al., (1972). Very low density lipoprotein-cholesterol was calculated according to Friedewalde, et al., (1972). The concentration was calculated from the following equation: VLDL-C concentration (mg / dl) = TG ÷ 5. Serum ALT and AST activites were determined according to the method of (Reitman and Frankel (1957); Enzymatic determination of urea was performed according to the method of Fawcett and Scott (1960) and creatinine was analysed by kinetic method kits described by Bartels et al., (1972).

**Statistical analysis:**

The obtained data was statistically analyzed using the Statistical Package for Social Science (SPSS) version 11.0 (SPSS , 1986).Values are represented as means with their standard errors. Unvaried analysis was
Conducted using analysis of variance for continuous variables. P value of less than 0.05 was considered to indicate statistical significance.

**Results**

Table (1): effect of Bifidobacterium and Lactobacillus acidophilus on glucose and insulin level in diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Glucose (mg/dl)</th>
<th>Insulin (miu/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (ve +)</td>
<td></td>
<td>203.80 ± 2.7 a</td>
<td>2.31 ± 0.28 e</td>
</tr>
<tr>
<td>Control (ve -)</td>
<td></td>
<td>90.80 ±2.5 d</td>
<td>4.29 ± 0.18 a</td>
</tr>
<tr>
<td>Lactobacillus</td>
<td></td>
<td>159.75 ± 8.3 b</td>
<td>3.10 ± 0.08 b</td>
</tr>
<tr>
<td>Bifidobacterium</td>
<td></td>
<td>144.75 ± 5.6 bc</td>
<td>3.36 ± 0.11 b</td>
</tr>
<tr>
<td>(Lactobacillus+Bifidobacterium)</td>
<td></td>
<td>139.75 ± 5.5 c</td>
<td>3.97 ± 0.02 a</td>
</tr>
</tbody>
</table>

- Values are expressed as means ± SE.
- Means with the different letter superscripts in the same column denote significance at P < 0.05.

Table (1) demonstrated the effect of Bifidobacterium and Lactobacillus acidophilus alone and in combination on glucose and insulin level in diabetic rats. Rats injected with streptozotocin induced hyperglycemia and hypoinsulinemia as compared to control negative group. Bifidobacterium and Lactobacillus acidophilus alone and in combination significantly decreased the mean value of serum glucose as compared to control groups. Bifidobacterium numerically decrease the value of serum glucose as compared to Lactobacillus acidophilus. In regarding to insulin, Bifidobacterium and Lactobacillus acidophilus alone and in combination significantly increased the mean value of serum insulin secretion as compared to control groups. Also, the combination between the two tested probiotic significantly increased the insulin secretion. It was observed that the combination between Lactobacillus acidophilus and Bifidobacterium was more effective than Bifidobacterium or Lactobacillus acidophilus alone in reducing the hypoglycemia and increasing insulin secretion.
Table (2) showed the effect of Bifidobacterium and Lactobacillus acidophilus on serum cholesterol and triglycerides level in diabetic rats. The results indicated that rats injected with streptozotocin induced hypercholesterolemia as compared to control negative group. Supplementation with Bifidobacterium and Lactobacillus acidophilus alone or in combination significantly decreased the mean value of serum total cholesterol and triglycerides as compared to control positive group. Also, there is no differences in the effect on total cholesterol and triglycerides between the two tested probiotic. The use of Bifidobacterium and Lactobacillus bacteria was more effective than the use of one.

Table (3) showed the effect of Bifidobacterium and Lactobacillus acidophilus on serum lipoproteins level in diabetic rats. The values are expressed as means ± SE. Means with the different letter superscripts in the same column denote significance at P < 0.05.
Table (3) illustrated the effect of Bifidobacterium and Lactobacillus acidophilus on serum lipoproteins level in diabetic rats. The results indicated that, supplementation with Bifidobacterium and Lactobacillus acidophilus alone or in combination significantly decreased serum low density lipoprotein cholesterol and very low density lipoprotein cholesterol as compared to control positive group, on the other hand, supplementation with Bifidobacterium and Lactobacillus acidophilus alone numerically increased serum high density lipoprotein cholesterol. Whoever, the combination between the two tested probiotic significantly increased the mean value of serum high density lipoprotein cholesterol as compared to control positive group.

Table (4): effect of Bifidobacterium and Lactobacillus acidophilus on serum kidney functions level in diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Urea (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (ve +)</td>
<td>57.80 ± 1.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.00 ± 0.009&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Control (ve -)</td>
<td>39.20 ± 1.65&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>0.92 ± 0.018&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Lactobacillus</td>
<td>42.25 ± 1.52&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.67 ± 0.037&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Bifidobacterium</td>
<td>37.25 ± 1.41&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>0.90 ± 0.031&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>(Lactobacillus +Bifidobacterium)</td>
<td>35.75 ± 1.31&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.86 ± 0.018&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

- Values are expressed as means ± SE.
- Means with the different letter superscripts in the same column denote significance at P < 0.05

Table (4) showed the effect of Bifidobacterium and Lactobacillus acidophilus on serum kidney functions level in diabetic rats. The results indicated that, supplementation with Bifidobacterium and Lactobacillus acidophilus alone or in combination significantly decreased the mean value of serum urea, uric acid and creatinine as compared to control positive group.
Table (5) showed the effect of Bifidobacterium and Lactobacillus acidophilus on serum liver functions level in diabetic rats. The results indicated that, Supplementation with Bifidobacterium and Lactobacillus acidophilus alone or in combination significantly decreased the mean value of serum AST and ALT as compared to control positive group. There is no differences between the two tested probiotic on the their effects on liver function , while the combination between Bifidobacterium and Lactobacillus acidophilus was more effective in improving liver function than either of them.

**Discussion**

Probiotics are dietary supplements containing potentially beneficial bacteria or yeasts. According to the currently adopted definition by FAO/WHO, probiotics are: ‘Live microorganisms which when administered in adequate amounts confer a health benefit on the host (FAO/WHO , 2001).’

Probiotics have been used for centuries in the form of dairy-based fermented products, but the potential use of probiotics as a form of medical nutrition therapy has not received formal recognition. Medical conditions that have been reportedly treated or have the potential to be treated with probiotics include diarrhea, gastroenteritis, irritable bowel syndrome, and inflammatory bowel disease (Crohn's disease and ulcerative colitis), cancer, depressed immune function, inadequate lactase digestion, infant allergies, failure-to-thrive, hyperlipidemia, hepatic diseases, Helicobacter pylori infections, genitourinary tract infections, and others (Brown and Valiere , 2004 ). Strains of the genera Lactobacillus and Bifidobacterium, are the most widely used probiotic bacteria (Tannock, 2005 ).
The results of our study indicated that, Bifidobacterium and Lactobacillus acidophilus alone and in combination significantly decreased the mean value of serum glucose and significantly increased insulin secretion as compared to control groups. These results were in agreement with (Yadav, et al., 2007) who found that, Lactobacillus acidophilus and Lactobacillus casei significantly delayed the onset of glucose intolerance, hyperglycemia, hyperinsulinemia and dyslipidemia. Also, Al-Salami, et al., (2008) indicated that, probiotic (75 mg/kg) for three days after which a gliclazide suspension treatment of diabetic rats increases gliclazide bioavailability and lowers blood glucose levels by insulin-independent mechanisms, suggesting that the administration of probiotics may be beneficial as adjunct therapy in the treatment of diabetes. Also, Hariom, et al., (2007) indicated that, the probiotic dahi-supplemented diet significantly delayed the onset of glucose intolerance, hyperglycemia, hyperinsulinemia, dyslipidemia, and oxidative stress in high fructose-induced diabetic rats, indicating a lower risk of diabetes and its complications.

Also, the results of our study showed that, Bifidobacterium and Lactobacillus acidophilus alone and in combination significantly decreased the mean value of serum total cholesterol, triglycerides, LDL-C and VLDL-C and significantly increased the mean value of serum HDL-C as compared to control groups. These results were in agreement with Lin and Chang, (2000) who showed that both intestinal strains (Bifidobacterium longum and Lactobacillus acidophilus) were able to protect plasma lipid from oxidation at different degrees. The inhibition rates on plasma lipid peroxidation ranged from 11 to 29% for 109 cells of B. longum and L. acidophilus. Generally speaking, B. longum demonstrated better antioxidative ability than L. acidophilus in this study.

Probiotic bacteria ferment food-derived indigestible carbohydrates to produce short-chain fatty acids in the gut, which can then cause a decrease in the systemic levels of blood lipids by inhibiting hepatic cholesterol synthesis and/or redistributing cholesterol from plasma to the liver. Furthermore, some bacteria may interfere with cholesterol absorption from the gut by deconjugating bile salts and therefore affecting the metabolism of cholesterol, or by directly assimilating cholesterol (Pereira and Gibson, 2002).

Animal studies have demonstrated the efficacy of a range of LAB to be able to lower serum cholesterol levels, presumably by breaking down bile in the gut, thus inhibiting its reabsorption (which enters the blood as cholesterol). Some, but not all human trials have shown that dairy foods fermented with specific LAB can produce modest reductions in total and LDL cholesterol.
levels in those with normal levels to begin with (Sanders, 2000). Supplementing rat diets with L. acidophilus ATCC 43121 increased bile acid deconjugation and dehydroxylation, resulting in increased cholesterol excretion. L. acidophilus ATCC 43121 had a negligible effect on cholesterol assimilation. These results indicate that the hypocholesterolemic effects of L. acidophilus ATCC 43121 are primarily the result of bile acid deconjugation and dehydroxylation (Microbiol and Biotechnol, 2007).

The results of our study showed that, Bifidobacterium and Lactobacillus acidophilus alone and in combination significantly improved liver functions as compared to control group. These results were in agreement with (Irina, et al., 2008) who reported that, patients with alcohol-induced liver injury have altered bowel flora compared to healthy controls. Short-term oral supplementation with bifidobacteria (7.9 vs. 6.81 log CFU/g) and lactobacilli (4.2 vs. 3.2 log CFU/g) was associated with restoration of the bowel flora and greater improvement in alcohol-induced liver injury than standard therapy alone. Also, Osman, et al., (2007) blueberry and probiotics exert protective effects on acute liver injury. They reduce the hepatocytes injury, the inflammation and the pro-inflammatory cytokines, and improve the barrier functions and antioxidant activity.

Our results were in agreement with Ranganathan, et al., (2005) who found that, probiotic dietary supplements caused a reduction in blood urea-nitrogen levels, concluding that supplementation of probiotic formulation to uremic rats slows the progression of azotemia, which may correlate with prolonged life span of uremic rats.

References
Adeghate E.;Ponery A.; Pallot D. and Singh J. (2001):
Al-Salami H.;Butt G.; Fawcett JP.; Tucker IG.; Golocorbin-Kon S. and Mikov M. (2008):
Bartels, H.; Bohmer, M.; Heierli, C., (1972):
“ Probiotics and medical nutrition therapy,
Recent Trends of Developing Institutional and Academic Performance in Higher Specific Education

Buccolo, G. and David, H. (1973):

FAO/WHO (2001):

Fawcett, K.K. and Scott, J.E., (1960):

Flegg, H. M. (1973):


Gupta, S., Kataria, M., Gupta, P.K., Murganandan, S., Yashroy, R.C., (2004):

Hariom Y.; Shalini J. and Sinha P.R. (2007):
"Antidiabetic effect of probiotic dahi containing Lactobacillus acidophilus and Lactobacillus casei in high fructose fed rats”. Nutrition. 23, (1) : Pages 62-68

"Effect of Dietary Inclusion of Lactobacillus acidophilus ATCC 43121 on Cholesterol Metabolism in Rats”. J. Microbiol. Biotechnol., 17(4), 655–662

Kostner, G. M. (1977):

“Antioxidative Effect of Intestinal Bacteria Bifidobacterium longum ATCC 15708 and Lactobacillus acidophilus ATCC 4356”. Digestive Diseases and Sciences, 45,(8) :1617–1622.


"The role of oxidative stress and NF (B) activation in late diabetic complications”. Biofactors 10, 171–179.
Effect of Bifidobacterium and Lactobacillus acidophi lus in diabetic rats


Nichols AW. (2007):


Pereira DI. and Gibson GR. (2002):
“Effects of consumption of probiotics and prebiotics on serum lipid levels in humans .


Reitman, S., Frankel, S., (1957):

SPSS (1986):
"SPSS-PC for the IBM PC/XT computer". Version 11.0. SPSS Inc., II. U.S.A.

Tinder ,P. (1969):

Tannock GW. (2003):

Sanders ME. (2000):
"Considerations for use of probiotic bacteria to modulate human health

Yadav H, Jain S, and Sinha PR. (2007):
تأثير البرويوبيوتاك على الحالة الغذائية للفقرات الصابنة بالسمر

علي السيد الحمسي

المつつ العربي

يعتبر الأنسولين والأدوية المخفضة للسكر والأخدة عن طريق الفم من السبل الرئيسية لعلاج مرض السكري وفعالية في السيطرة على ارتفاع السكر في الدم، ولكن هذه الأنواع من الأدوية أيضاً لـLactobacillus و Bifidobacterium آثار جانبية واضحة. ولهذا اجتذبت بكتريا البرويوبيوتاك مثل Bifidobacterium الكثير من الاهتمام لأنها تحمل في إطار الصحة البشرية. تهدف هذه الدراسة إلى تأثير عسل من Lactobacillus acidophilus و Bifidobacterium في تقليل الفقرات الصابنة بالسمر 10⁸ cfu/day بمدرينها أو مجتمعين على سكر الدم في الفقرات الصابنة بالسمر وملاحظة معرفة تأثيرهما على وظائف الكبد والكلي.

تم تقسيم 30 ذكر بن (120 ± 5 جرام) بطريقة عشوائية إلى 5 مجموعات متساوية كالمجموعة الأولى وتمثل مجموعة الكنترول السالبة وتم تقديم على الوضعية الأساسية فقط. المجموعات من الثانية حتى الخامسة مصابة بالسمر، المجموعة الثانية تمثل مجموعة الكنترول الموجبة وتم تقديم على الوضعية الأساسية فقط. المجموعة الثالثة وتمثل مجموعة الكنترول السلبية وتم تقديم بكتريا Bifidobacterium لـ 10 طرق بدء، المجموعة الرابعة تأخذ بكتريا Lactobacillus acidophilus و Bifidobacterium لـ 10 طرق بدء، المجموعة الخامسة تأخذ خليط من ما تم تجميع عينات الدم من الفقرات وفصل السيرام لتحليل مستوي الجلوخوز الأنسولين صورة الدهون ووظائف الكبد والكلي.

Bifidobacterium و Lactobacillus acidophilus و Lactobacillusacidophilus و Bifidobacterium و Lactobacillus acidophilus و بكتريا Bifidobacterium و Lactobacillus acidophilus ممثلاً وعديداً من البرويوبيوتاك بدرجة معنوية تختفي مصطلحات الكيتان والليسيبرين المخفضة والكثافة والليسيبرينات المخفضة الكيتان جداً كما إتاحة استخدام عينات الدم من الفقرات وفصل السيرام لتحليل مستوي الجلوخوز الأنسولين وبنية الكبد والكلي. الأساسية ومعرفة تأثير الفاكهة من استخدامها بمفردات.

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