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Research Article

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Lactobacillus species Probiotic Improves the Metabolic Syndrome Associated Disorders Induced in Rats

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ABSTRACT

Animals fed a high-fructose diet developclinical characteristics of metabolic syndrome; therefore,high-fructose fed animals are particularly useful for assessingpotential therapeutic interventions against metabolic syndrome.In the present study we investigated the effect of Lactobacillus LP on hyperglycemia, insulin resistance and hypertriglyceridemia in fructose drinking rats as a model of metabolic syndrome. Male albino rats were given a15%fructose solution as drinking water for 13 weeks. Fructose solution significantly increased the serum concentrations of glucose, triacylglycerols, VLDL-c and LDL-c in comparison with control group. It also induced insulin resistance which expressed by HOMA-IR. Administration of lactobacillus LB significantly improved the levels of serum glucose,triacylglycerols, VLDL-c and LDL-c by (36%, 40.2%, 40.2% and 50%) respectively. It also significantly improved the HOMA-IR by 56%. These results suggested the protective role of lactobacillus LB against the metabolic syndrome associated disorders like hyperglycemia, insulin resistance and hyperlipidemia.

Keywords*: metabolic syndrome, high fructose, probiotics.*

INTRODUCTION

Metabolic syndrome diagnosis implies in positive results to at least three metabolic alterations including insulin resistance, hypertension, obesity, endothelial dysfunction and blood lipid profile alterations[28]. There has been a heightened awareness of the metabolic syndrome and a subsequent increase in clinical attention directed towards prevention due to its strong association with premature morbidity and mortality[29]. Metabolic syndrome affects more that 25% of population in the developed and underdeveloped world with an associated threefold increased risk for cardiovascular mortality. It is therefore critical to identify mechanisms and strategies to prevent or treat it[3].

Consumption of calories, and specifically of refined carbohydrates and fructose, is clear and correlates positively with an increases in metabolic syndrome. In animal models, diets high in fructose induce features of the metabolic syndrome including weight gain, insulin resistance, hypertriglyceridemia, and hypertension. Similar effects are observed in humanswith the consumption of fructose-sweetened beverages[23].

Probiotic is a live microbial culture or cultured dairy product, which plays a fundamentally important role in health and disease[15]. Probiotics are safe and widely accepted by the public. Over the past five years, probiotics have rapidly emerged as natural therapeutics with potential to target key risk factors associated with metabolic syndrome[5]. Therefore, the aim of the present study was to evaluate the antidiabetic and antilipidemic effects of Lactobacilli species probiotic.

MATERIALS AND METHODS

Chemicals

Pure D (-) fructose (Laevulose) was purchased from Al Nasr pharmaceutical chemicals Company, Egypt.Lacteol fort was obtained from Rameda Company, Egypt under license of Axcan pharma S.A. - France. All the other chemicals were of the highest analytical grade and purchased from Sigma-Aldrich Company.

Experimental animals and protocol

Sixty male Albino rats of 250 ± 30 g B.W range were purchased from National Research Centre, Dokki, Giza. They were housed individually in metallic cages at a room temperature of 22±1°C under a 12-h light- dark cycle. Our studies were carried out in accordance with the regulations for the use and care of experimental animals according to faculty of veterinary medicine, Beni-Sueif University.After one week of acclimatization, rats were randomly divided into four groups, which were control group (Cr), fructose group (F), fructose +lactobacilli group (FL) and lactobacilli group (L). Rats in all groups were maintained on standard rat chow diet.

Rats in control group were maintained on normal tap drinking water during the all period of the experiment (13 weeks). Rats in the fructose group (F) were maintained on 15% fructose solution (75 gm fructose added to 500 ml water) daily in a free manner during the all period of experiment[27]. Rats in the fructose +lactobacilli group (FL) were maintained on 15% fructose solution plus lacteol fort probiotic solution which composed of Lactobacillus LBcorresponding to Lactobacillus delbrueckii and Lactobacillus fermentum 10 billion (one sachet of lacteal fort added to 500 ml of water) daily in a free manner during the all period of experiment[10]. Rats in Lactobacilli group (L) were maintained on lacteal fort probiotic solution as previous group.

Collection and processing of samples

At the end of experiment (after 13 weeks), serum blood samples were separated after overnight food fasting. Serum samples were divided into several aliquotsand were kept at -20 C˚ for analysis of different biochemical measurements.

Blood biochemical analysis

Fasting serum glucose level was determined according to the enzymatic method of Trinder (1969) by using of commercial diagnostic laboratory kit (Spinreact, Giza, Egypt). Serum triacylglycerols, serum total cholesterol, serum HDL-cholesterol were determined by enzymatic colorimetric method according to Fassati and Prencipe (1982), Richmond (1973) and Burstein *et al*. (1970) respectively and by using of commercial diagnostic laboratory kit (Bio-diagnostic company, Cairo, Egypt). Serum TC-LDL and TC-VLDL were calculated according to Friedewald *et al*., formula (1972)

TC-LDL = TC – TC-VLDL (TG/5) – TC-HDL.

Fasted serum insulin hormone level was determined by ELISA method according to Kjems *et al.*, (1993) by using of insulin microplate ELISA test (Monobind Inc., Lake Forest, USA). Insulin resistance was calculated by using of Homeostatic model assessment formula as described by Metthewset al. (1985).

HOMA- IR=[fasting serum glucose $_{(mg/dl)}$ x fasting serum insulin $_{(\mu IU/ml)}$] ÷ 405

Statistical analysis

Statistical analysis was carried out using GraphPadInstat software (version 3, ISS-Rome, Italy). Unless differently specified, groups of data were compared with un-paired t-test and one-way analysis of variance (ANOVA) followed by Tukey-kramer (TK) multiple comparisons post-test. Values of $P<0.05$ were regarded as significant. The data, as clearly indicated are reported in tables and figures as mean ± standard error (S.E).

RESULTS

Effect of Lactobacillus LB probiotic on serum concentrations of glucose, insulin and insulin resistance in different rats groups.

Results in table (1) showed a significant increase in serum concentration of glucose and in HOMA index of insulin resistance in F-group in comparison to Cr-group. Serum insulin concentration was non-significantlyincreased in F-group. lactobacillius LB administration significantly decreased and improved the previous levels by 36%, 56% and 12.9% respectively indicating its hypoglycemic effect.

Table (1) fasting serum glucose concentration (mg/dl), fasting serum insulin concentration (pmol/l) and calculated HOMA-IR of the control group, F-group, FL -group and L-group at the end of the experiment (W13)

	Cr-group	F- group	FL- group	L-group	% of improvement
Fasting serum glucose (mg/dl)	70.75 ± 11.57	135 ± 25.43 [a]	68.43 ± 6.72 [b]	83.56 ± 13.42	36%
Fasting serum insulin (µIU/l)	12.3 ± 0.71	14.77 ± 1.05	12.87 ± 0.72	13.1 ± 0.38	12.9%
HOMA-IR	2.15	4.92[a]	2.17[b]	2.7	56%

Values of serum glucose and serum insulin are statistically analyzed by one way ANOVA test and followed by Tukey-kramer post-test and reported as mean±S.E.
[a] vs control at *p< 0.05, [b] vs fructose, % of improvement of FL-group compared to F- group
Values of HOMA-IR are explained by Matthews's HOMA score.

Effectof Lactobacillus LB probiotic on serum concentrations of TAC and TC in different rats groups.

Results in table (2) showed a significant increase in serum concentration of TAG of F-group in comparison to Cr-group indicating hypertriglyceridemia. Both FL-group and L-group showed a significant decrease compared to F- group indicating the hypolipidemic effect of lactobacillius LB. The percent of improvement was 40.2%.The slight increase in serum cholesterol was not significantly varied either in comparison with Cr-group or F-group, while there was a slight improvement (4.8%) due to lactobacillius LB administration.

Table (2) Serum triacylglycerols and total cholesterol concentrations (mg/dl) of the Cr-group, F-group, FL-group and L-group at the end of the experiment (W13)

	Cr-group	F- group	FL- group	L-group	% of improvement
Serum TAG (mg/dl)	108.72± 13.55	393.76 ± 20.76 [a]	235.54 ± 18.74 [b]	186.69 ± 12.64 [b]	40.2%
Serum TC (mg/dl)	93.56 ± 6.73	111 ± 6.36	105.66 ± 5.55	92.62 ± 13.13	4.8%

Values are statistically analyzed by one way ANOVA test followed by Tukey-Kramer post-test and reported as mean±S.E.
[a] vs control and [b] vs fructose at P< 0.001, % of improvement of FL- group compared to F-group.

Effect of Lactobacillus LB probiotic on serum concentrations of different lipoprotein fractions in different rats groups.

Results in table (3) showed a significant increase in serum concentration of both VLDL-c and LDL-c in the F-group in comparison to Cr-group.lactobacillius LB administration significantly decreased this elevation by 40.2%nd 50% respectively in comparison to F-group. Serum concentration of HDL-c was decreased in F-group but not significantly in comparison to Cr-group. LDL/HDL ratio was significantly increased in F-group in comparison to Cr-group indicating atherogenic index which was significantly improved in FL-group by 51.4%.

Table (3) Cholesterol concentration (mg/dl) of serum lipoprotein fractions of the Cr-group, F-group, FL-group and L-group at the end of the experiment (W13)

	Cr-group	F- group	FL- group	L-group	% of improvement
calculated VLDL-c	36.11 ± 2.72	78.75 ± 4.15 [a]	47.11 ± 3.75 [b]	37.34 ± 2.53 [b]	40.2%
calculated LDL-c	77.79 ± 8.45	244.61 ± 18.55 [a]	122.3 ± 17.5 [b]	94.35 ± 14.62 [b]	50%
HDL-c	66.81 ± 4.42	60.4 ± 5.66	66.13 ± 5.37	55 ± 6.18	9.5%
LDL /HDL Ratio	1.16 ± 0.2	3.7 ± 0.3[a]	1.8 ± 0.3 [b]	1.7 ± 0.4	51.4%

Values are statistically analyzed by one way ANOVA test followed by Tukey-Kramer post-test and reported as mean±S.E.

[a] vs control, [b] vs fructose at P< 0.001, % of improvement of FL-group compared to F-group.

DISCUSSION

Many studies use high fructose ingestion for induction of metabolic syndrome and its associated disorders but with some differences. These differences are attributable mainly to differences among experimental protocols. In our experiment, administration of 15% fructose solution to rats daily for 13 weeks succeeded to induce a significant increase in fasted serum glucose level (hyperglycemia) and mild non-significant increase in fasted serum insulin concentration as showed in table (1). While calculated HOMA-IR showed moderate insulin resistance compared to control group. These results are similar to that reported by Leibowitz *et al.*[13] as feeding of Sprague-Dawely rats with fructose enriched diet (60%) for 8 weeks showed normal insulin level in the blood. The most acceptable explanation of these results is reported by Basciano *et al.*[1]. They clarified that fructose does not appear to acutely increase insulin secretion (normal insulin level) as it is non-insulin dependent, but cause hyperglycemia and insulin resistance through other mechanisms. Insulin resistance is closely linked to lipid metabolism disorders (Dyslipidemia); more specifically, insulin-resistant subjects have higher ectopic lipid deposition, which may generate lipid-derived metabolites, such as diacylglycerol, fatty acyl CoA, and ceramides. The presence of these metabolites in the intracellular environment leads to a higher phosphorylation of insulin receptor substrate-1 (IRS-1), which has been shown to reduce insulin signaling causing insulin resistance[24]. Visceral adiposity is known to be increased by high fructose intake and it is associated with IR. The greater lipolytic capacity of visceral than peripheral adipocytes releases more FFAs to the portal circulation. Increased amounts of FFAs directly affect insulin signaling, diminish glucose uptake in muscle, and induce gluconeogenesis in the liver[17].

Results recorded in table (1) showed the antidiabetic effect of lactobacillus LB as it improved hyperglycemia and insulin resistance by about 36% and 56% respectively (table 1). Our results were supported by that recorded by Yadav *et al.*[26] and Hariom *et al.*[8] as they reported that using of fermented milk product containing L. acidophilusand L. casei delayed the progression of high fructose-induced hyperglycemia, hyperinsulinemia, dyslipidemia, and oxidative stress in rats.

Hypertriglyceridemia is achieved in our experiment (table 2) as fasted serum TAC was significantly increased in the F-group compared to Cr- group and also there was a mild increase in fasted serum cholesterol level but this increase was not statistically significant (table 2). Our reported results are in accordance with the results obtained by Padiya *et al.*[19] Zamami *et al.*[27], Kumamoto *et al.*[12] and Mohammadi *et al.*[18]. Fructose consumption has been suggested to induce hypertriglyceridemia through both increased hepatic TAG that can be packed into very-low density lipoproteins by the liver and reduced TAG clearance by adipose tissue[21]. Administration of lactobacillus LB succeeded to improve the previous result as reported in table (2) by 40.2% for serum TAC level and by 4.8% for serum TC level, indicating its hypolipidemic effect[10]. In our experiment, there was a significant increase of both serum levels of VLDL-c and LDL-c in F-group compared to Cr-group also LDL-c/HDL-c ratio increased three times than that of Cr-group (table 3).

These results supported by El Mesallamy *et al*.[4] and Shahraki *et al*.[22] as administration of 10% fructose solution to male Wister rats for 8 weeks lead to increased serum levels of both VLDL and LDL without any change in HDL level. As results recorded in (table 3) lactobacilli LB improved high serum levels of LDL-c and VLDL-c induced by high fructose solution by about respectively 50% and 40.2% in a significant manner while improved HDL-c by about 9.5% and LDL/HDL Ratio was improved strongly by 51.4% compared to F-group. That was augmented by (Hsieh et al., 2013)as they found that oral administration of Lactobacilli bacteria lowered LDL and TG serum levels in high fructose fed rats for 14 weeks. A study by (Lye et al., 2010) showed that there existed many possible probiotic mechanisms lowering Cholesterol Specially LDL-c including assimilation of cholesterol during growth, binding of cholesterol to cellular surface, disruption of cholesterol micelle, de-conjugation of bile salt and bile salt hydrolase activity.

CONCLUSION

It could be concluded that, the administration of fructose solution (15%) to male albino rats for a period of 13 weeks, induced hyperglycemia, insulin resistance, hypertriglyceridemia and increased serum LDL-c, VLDL-c, concentrations respectively. According to the hypolipidemic and antidiabetic effect of lactobacilli LB, they improved hyperglycemia, insulin resistance, hypertriglyceridemia and decreased serum concentrations of LDL-c and VLDL-c. Administration of lactobacilli LB probiotic solution to normal rats caused some adverse effects as somewhat increased fasting serum glucose level and triglyceride level. These points need more researching efforts to be explained.

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