Anti Obesity and Lipid - Lowering Effect of Lactobacillus Spp. as Probiotic on the Obese Rat.

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Abstract:
The effects of Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus casi and Lactobacillus rhomunas on the body weight and lipid metabolism on obese rats were evaluated. In the body weight, the results showed significant deference between rat groups, the group that consumed 3 probiotic strains showed reduction in the body weight with ratio 16.63% while the group consumed 2 probiotic strains showed decreasing with ratio 17.62% compared with control group which increased in the body weight.In the effeci of probiotics on lipid metabolisim the results showed significant deference between rat groups, the group that consumed 3 probiotic strains showed decreasing in the cholesterol with ratio 50.7%, triglyceride with ratio 48.8 %, LDL with ratio 51% and elevated of HDL level with ratio 45%, while the group consumed the 2 probiotic strains showed decreasing in the cholesterol with ratio 48.3%, triglyceride with ratio 43.3 %, LDL with ratio 27.4% and elevated HDL level with ratio 26%. The study suggests that LAB supplementation has hypocholesterolemic and anti obesity effects in rats. These strains might be able to improve the intestinal microbial balance and potentially improve intestinal transit time.
Key word: lipid, anti obesity, probiotic.

تاثير المعزز الحياتى .Lactobacillus spp كمضاد للسمنة وخافض للدهون لدى الجرذان السمينة

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الخلاصة
درس تاثير اربع انواع من بكتريا اللاكتوباسلس وهى: Lactobacillus acidophilus,Lactobacillus plantarum, Lactobacillus casi and Lactobacillus rhomunas وتم تقيم تاثيرها على وزن الجسم وايض الدهون لدى الجرذان.اظهرت النتائج ان التاثير على وزن الجسم فروقات معنوية بين المجاميع حيث اظهر ت المجموعة التى استهلكت معززات حيوية ذات ٣ انواع من البكتريا نقصان فى معدل الوزن بمقدار ١٦,٦٣% بينما اظهرت المجموعة الثانية التى استهلكت معززات حيوية مع نوعين من البكتريا نقصان فى الوزن بمعدل ١٧,٦٢% مقارنة مع مجموعة السيطرة التى اظهرت زيادة فى الوزن. وعند دراسة تاثير المعززات الحيوية على الدهون اظهرت الدراسة فروقات معنوية واضحة بين المجاميع اظهرت المجموعة التى استهلكت المعزز الحيوى ذى الثلاثة انواع نقصان فى معدل الكولسترول بمقدار ٥٠,٧% وفى الدهون الثلاثية بقدار ٤٨,٨% وفى الدهون الضارة بقدار ٥١% واظهرت زيادة فى الدهون النافعة بمقدار ٤٥% واظهرت المجموعة الثانية نقصان الكولسترول بمقدار ٤٨,٣% وفى الدهون الثلاثية ٤٣,٣% وفى الدهون الضارة ٢٧,٤% وزيادة فى نسبة الدهون النافعة بنسبة ٢٦%. استنتجت الدراسة ان المكملات الغذائية الحاوية على بكتريا الحليب تلعب دورا فى السيطرة على ارتفاع الدهون والسمنة فى الجرذان. هذة الدراسة تبين مدى الدور الذى تلعبة البكتريا المعوية فى موازنة عمليات الايض فى الجسم.

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Introduction

Elevated serum cholesterol level is widely recognized as a contributory risk factor for the development of cardiovascular diseases (CVD) such as atherosclerosis, coronary heart disease and stroke‹ The World Health Organization (WHO) has predicted that by 2030, CVD will remain the leading causes of death and affect approximately 23.6 million people globally [1].It has been reported that even a 1% reduction in serum cholesterol could reduce the risk of coronary heart disease by 2-3% [2]. Current drug therapies, with their high relative costs and associated side effects, are not viewed to be the optimal long-term answers. The development of alternative management strategies for the treatment of hypercholesterolemia is necessary, especially for people with borderline cholesterol levels. Since Mann and Spoerry [3] discovered the hypocholesterolemic effects of fermented milk ingested by the Massai tribes people, the relationship between lactic acid bacteria (LAB) and the serum cholesterol has become a focus of great interest. Studies evaluating this relationship have found that Lactobacilli or Bifidobacteria can exhibit hypocholesterolemic properties in animal models [4] and in humans. [5]. Several hypotheses have been proposed to explain these findings: (**1**) consumption of cholesterol by intestinal bacteria, thus reducing the amount of cholesterol available for absorption[6]; (**2**) cholesterol may be bound to the bacterial cellular surface [7] or incorporated into the bacterial cellular membranes [8] or converted into coprostanol by cholesterol reductase, which is produced by strains of lactobacilli [9] (**3**) inhibition of micelle formation by certain probiotic strains [10] (**4**) short-chain fatty acids produced upon selective fermentation of food by intestinal bacterial microflora may lower plasma cholesterol levels [11] (**5**) some bacterial species excrete bile salt hydrolase, leading to increased bile excretion in feces [12] However, other reports are contradictory and fail to show hypocholesterolemic effects of probiotics [13] Consequently, this area remains controversial. Therefore, more information is required to strengthen the proposed hypotheses and improve our understanding of how bacteria affect cholesterol metabolism, which might lead to more appropriate use of probiotics. This study aimed to examine the effects of LAB strains on cholesterol levels and body weight.

Methods

Probiotic strains

Four probiotic strains were used this study (L.acidophilus, L. Casei, L. Rhamnosus, L. Plantarum), were produced by Vitane Pharma-GmbH_Germany .To re identification of strains, biochemical tests were used [14].

Study design:

The study was designed as a permuted blocked randomized trial. There were three parallel groups and the study was conducted for 4 weeks. Each group of rats containing 7 rats age between 9 month to 12 month,weight for first group between 460 - 400 (g) and second group from 380_300 (g) and control group weight from(250_200)g .The first group consumedprobiotics with three bacterial strains(L.acidophilus, L. Casei, L. Rhamnosus),the second group consumed probiotic strains with two strains of bacteria(L.acidophilus, L. Plantarum) twice daily for 4 week, in the morning and afternoon, the third group was consumed basal diet. Weight of all groups were measured before the study and at the end of study, in the end of 4 weeks blood of all group were collected for measuring lipid profile. Animals were kept under suitable environmental conditions such as room temperature (24-26 C˚) exposed to light 14 hr. /day and were fed the basal diet. The diet was locally made of components mentioned in table1 [15].

The dose of probiotics administrated was 0.5 ml two times per day for 4 weeks. Control and model groups received an equivalent amount of normal saline. Body weight was recorded weekly and food consumption was monitored daily.

Assay for serum lipids

Blood sample (4 mL) was obtained from the celiac vein and transferred to nonheparinized vacuum collection tubes. Tubes were initially held stationary at 0°C for 30 minutes, and then centrifuged at 2000 × g for 15 minutes at 4°C. Serum total cholesterol (TCH), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and triglycerides (TG) were measured by commercial kits (**Spin react-Spain).**

Statistical Analysis

The Statistical Analysis System- SAS (2004) was used. The least significant difference (LSD) test was employed to compare means in this study. The usual methods, which used in order to analysis and assess the results, they

include: Descriptive statistics: a- Statistical tables. b- Graphic presentation.

Result and discussion

The results of the effect of probiotic strains on the body weight showed significant differences ($p<0.05$) between three groups table(2). The probiotic with three strains and two strains were effective on the body weight and caused significant decrease (($p<0.05$) in the weight of animal compared with control group which showed significant differences in weight gain figure 1.

Table 1- Effect of probiotic strains on the body weight in animals

Group	Body weight Mean ± SE(g)		
	Before consuming	Mid. Consuming	After consuming
Control	231.67 ± 5.69 C	268.33 ± 5.16 C	285.83 ± 2.82 B
Consuming 3 probiotic strains	415.42 ± 16.71 A	368.28 ± 16.24 A	346.33 ± 1815 A
Consuming 2 probiotic strains	343.71 ± 15.08 B	309.71 ± 12.92 B	283.14 ± 11.54 B
LSD Value	41.91 * P<0.002**	38.63 * <0.015**	37.44 * 0.0036**
P < 00.5*			

The probiotic strains showed a significantly effect the body weight; the rats group that consumed 3 probiotic strains showed 16.63% reduction in the body weight While the group that consumed 2 strains probiotic showed 17.62% reduction. These Results agree with a recent study [16] which represented the role of probiotic in treatment of obesity.

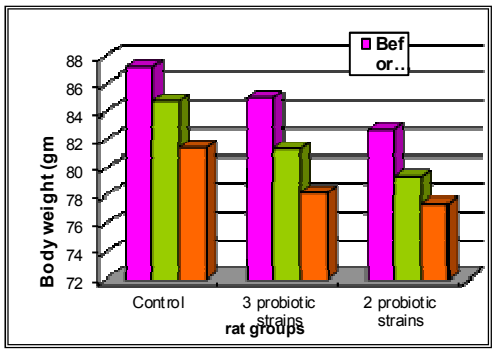


Figure 1- The effect of probiotic strains on the body weight in rats

Lee et al. (2006) reported that the L. plantarum and L. paracasei were associated with normal weight in culture of experimental models and the L. Plantarum had anti obesity effect in the mice[17]. Other Lactobacillus strains have shown an antiobesity effect in animals and humans similar to the L. gasseri SBT2055 (LG2055) strain in lean Zucker rats [18].This anti obesity effect may be linked to the production of specific molecules that can interfere with host metabolism, such as conjugated linoleic acid (CLA) for L. plantarum or L. Rhamnosus [19].

Effect of probiotic strains on the lipid profile

The serum cholesterol, HDL, LDL and triglyceride levels in the three rat groups were shown in table 3. Cholesterol and LDL and triglyceride levels differed significantly among these three groups. Rats fed 3 strains of LAB showed greatly decreasing of serum cholesterol, LDL and triglyceride levels in compared with control group ($p < 0.05$). The probiotic strains displayed in vivo hypocholesterolemic abilities, the 3 probiotic strains achieved a maximal cholesterol reduction by 50.7% ;57.71 ± 3.51 vs. 113.8 ± 3.20 mg/dL ($p < 0.05$), in the two probiotic strains system the cholesterol reduction was 48.3%;55±6.7vs. 113.8 compared with control as it depicted in figure 2.

Table 2- Effect of probiotic strains on the lipid profile in animals

Lipids	Control (Mg/dl)	Consuming with 3 probiotic strains (Mg/dl)	Consuming with 2 probiotic strains (Mg/dl)	LSD Value
Cholesterol	113.80± 3.20 A	57.71±3.51B	55.00±6.7 B	13.750*p <0.0034**
Triglyceride	100.80± 2.26 A	49.28±3.57B	43.67±8.29B	16.671*p <0.013**
HDL	54.6± 2.26 A	79.2±2.41B	68.9±2.93 B	12.231*p <0.0116**
LDL	17.32 ± 2.08 A	8.85±0.89B	4.76 ±1.40 B	4.291* p <0.047*

- (P<0.05), NS:Non-significant
- * Means having different letters at the same row are significantly different.

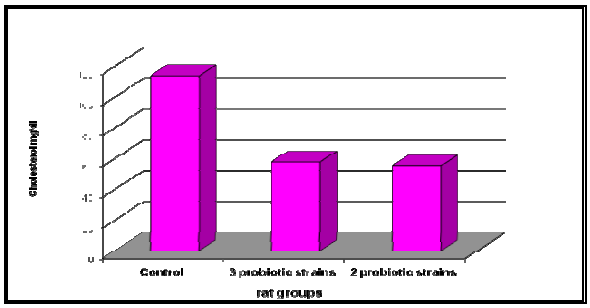


Figure 2- Effect of probiotic strains on cholesterol level.

The serum triglyceride levels were significantly lowered in the probiotic consumed group, the reduction of three strains consuming group was 48.8%; 49.28 ± 3.57 vs. 100.80. ± 3.20 mg/dL ($p < 0.05$) while the 2 strains consuming group displayed a triglyceride reduction of 43.3% ; 43.67 ± 8.29 vs. 100.8. ± 2.26 mg/dL, $p < 0.05$) compared with control group figure 3.

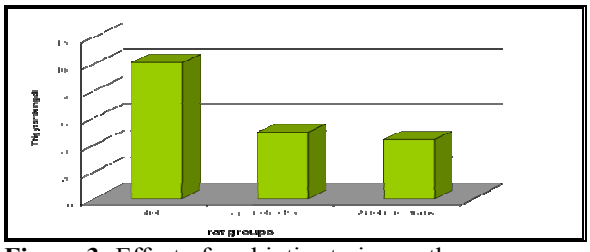
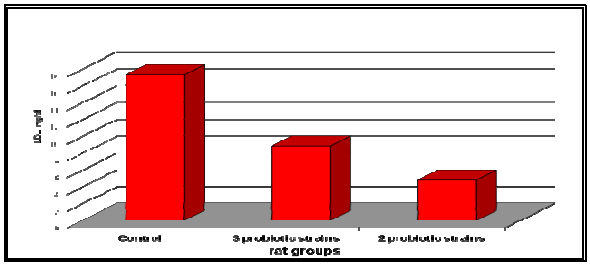


Figure 3- Effect of probiotic strains on the triglyceride after treatment.

The LDL level were reduced by 51 % (8.85 ± 0.89) vs. (17.32) mg/dL, ($p < 0.05$) in the 3probiotic strains group, while 2 probiotic strains group showed reduction of 27.4%(17.3.2±4.76) compared with control group figure 4**.**



Figures 4- Effect of probiotic strains on the LDL after treatment.

The effect of probiotic strains on the HDL(good lipids) was significant when 3 strain consumed group showed increased by 45% ; 79.2 mg/dL vs 54.6. mg/dl ($p < 0.05$), while 2 strains consumed group showed increased by 26% ; 68.9 vs 54.6 mg/dl ($p < 0.05$) compared with control group figure 5.

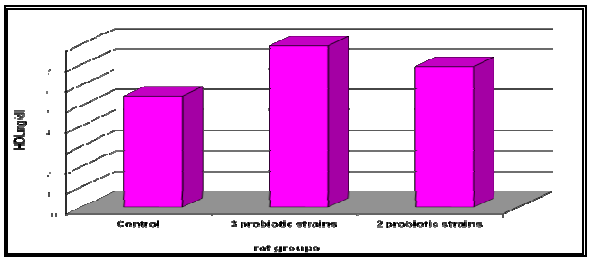


Figure 5- Effect of probiotic strains on the HDL level.

Hypocholesterolemic effects of probiotic strains or elevated level of total cholesterol in the bloodstream, is the result of high levels of low-density lipoprotein (LDL) as compared to high-density lipoprotein (HDL) [20]. Many Lactobacilli, being the natural inhabitants of the intestine, possess bile-salt hydrolase activity. This property has been used for developing probiotic formulations to combat hypercholesterolemia [21] .Many animal models have been used to evaluate the effects of probiotics on serum cholesterol levels [22].

Abd El-Gawad used buffalo milk yogurt fortified with Bifidobactirium longum in male albino rats for 35 days, total cholesterol was reduced by 50%, LDL-cholesterol by 56%, and triglycerides by 51% in comparison to the control [23] .When Lactobacillus plantarum PH04 was evaluated for its cholesterol lowering effects in rats, the total serum cholesterol and triglyceride levels showed a significant reduction as compared to the control [24].

Conclusion

From the study, the following can be conducted:The probiotic strains showed lowering effects on abdominal adiposity, body weight and other measures of subjects with obese tendencies, suggesting its beneficial influence on metabolic disorders.

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