International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.10 No.6, pp 20-24, 2017

The Effect of Probiotic (*Lactobacillus acidophilus*) on the Absorption of Calcium and Cholesterol in The intestines of Rats

Mohammed karee jabbar*

Faculty of pharmacy, Humanity of studies university college, Iraq.

Abstract : The interaction of Lactobacillus acidophilus with some physiological aspects of intestinal in regard to intestinal mucosal cells proliferation , differentiation and absorption were studied . The present study designed to study the influence of LBA on intestinal absorption. Therefore, male Swiss albino rats and divided into two main groups of rats each group consist of five rats , - first group: this Control (C)group received (1 ml per animal)of distilled water by oral gavages tube for 4 week . Second group: this treated(T) group received (5*10 CFU) Lactobacillus acidophilus as probiotics by oral gavages tube for 4 week and eachgroup was fed on a balanced diet with high- calcium (1.8%) from calcium citrate.The experiment continued for 30 days . At the end of experiments, all experimental animals were sacrificed .Serum levels of calcium and cloistral measurement.
Results of the present experiment revealed that there was significant difference in levels of calciumin C,T and the levels of calciumwhich was significantly($p<0.05$) increased in rats of T compared with C ,the results showed significant decreasein lipid profile ($p<0.05$) in T group compared with C group.

Introduction

Cardiovascular disease due to atherosclerosis of the arterialvessel wall and to thrombosis is the foremost cause ofpremature mortality and of disability-adjusted life years inEurope and is also increasingly common in the developingcountries [1]. Hyperlipidaemia is a dominant risk factorfor cardiovascular diseases and the leading cause of deathin many countries. Elevated serum cholesterol is generallya risk factor correlated with the development of coronaryartery diseases. Dietary fat is one of the most importantenvironmental factors associated with the incidence of thosediseases; diets high in cholesterol and saturated fat havebeen shown to promote atherosclerosis [2]. Atherosclerosisis considered to be a modified form of chronic inflammationinduced by lipids and many have followed in this pathincluding evidence that numerous cell adhesion moleculesand growth factors were determined in the atherosclerоticplaques [3]. The current drug therapy has the disadvantage owing to its undesirable side effects and cost, so there isan increasing interest in alternative approaches to lowercholesterol [4]. Diet intervention supplements have nowbeen extensively studied to reduce risk factor for cardiovasculardisease. Numerous animal experiments and humanstudies have reported that probiotic microorganisms display hypolipidemic effects by inhibiting cholesterol biosynthesisand decreasing low density lipoproteins [5, 6]. Food andAgriculture Organization and World Health Organization defined probiotics as “Living microorganisms which whenadministered in adequate amounts confer a health benefiton the host” [7]. There is currently adopted definition by FAO/WHO, but in the present research publications there arealso new definitions of probiotics as follows: probiotics areliving microorganisms which

modulate the specific functionof organism by activation of specific molecular pathways [8].The gastrointestinal tract is one of the largest interfacesbetween the outside world and the human or animal internalenvironment.

II. Material and Methods

Ten health Albino Wister pregnant male rats, were kept under suitable condition of (21-25 C) in an air condition room ,they were fed freely with standard pellet diet .The animals were divided into two main groups. **first group**: this Control (C) group received (1 ml per animal)of distilled water by oral gavages tube for 4 week . **Second group**: this treated (T) group received (5*10 CFU) Lactobacillus acidophilus as probiotics by oral gavages tube for 4 week . **And** each group was fed on a balanced diet with high- calcium (1.8%) from calcium citrate .

Table 1. Diet composition (%) of experimental rats

Component	Control	Subgroup 1	Subgroup 2	Subgroup 3
Proteins	20	20	20	18.7
Fats	10	4	4	4
Carbohydrates	60	66	60.75	54.8
Cellulose	5	5	5	5
Minerals	4	4	4	4
Vitamins	1	1	1	1
Calcium citrate	---	---	5.25	---
Dry skimmed milk	---	---	---	10
Hydroxyapatite	---	---	---	2.5

Table 2. Composition of mineral mixture (100g)

Component	Amount in gram
$CaCO_3$	20
K_2HPO_4	52.8
$CaHPO_4.2H_2O$	6
$MgSO4.7H_2O$	8.16
NaCl	10.08
$Fe(C_6H5O_7).7H_2O$	2.2
KI	0.064
$MnSO_4.4H_2O$	0.4
$ZnCl_2$	0.02
$CuSO_4.4H_2O$	0.024

Diet composition (%) of different rat subgroups is shown in Tables (1- 2).

At the end of the experiment all the experimental animals were anesthetized by intraperitoneal injection with 35 mg/kg ketamine hydrochloride. After opening the chest cavity, blood was collected by acupuncture through the left ventricle. Blood samples were collected twice; at the beginning and atthe end of experimental period (after 4 weeks). The first bloodsamples were collected from Jugular vein of rats. Secondblood samples were collected from animals‟ hearts after anesthesiaand sacrificing at the end of the 4^{th} week.

Laboratory Analysis

1-Determination of lipid profile in serum

The blood serum was used for determination of total cholesterol (TC), high density lipoproteins cholesterol (HDL-C), and triglycerides (TG) were measured by using an automatic biochemical analytical system. Low density lipoprotein (LDL-C) was calculated byFriedewald formula (W. T. Friedewald et al., 1982)

2-Determination of calcium in serum

Serum calcium was determined according to the method ofGindler and King (1972).

a. Principle

1. Calcium ion produces with methylthymol blue, in an alkalinemedium, a blue color the intensity of which is in proportion to40
2. the calcium concentration. The presence of hydroxyl 8 –quinoline eliminate the interference due to magnesium ions.

b. Reagents

1. Standard: (10 mg/dl =2.5 mmol/l).

2. Chromogen :methylthymol blue (0.2mmol/l),hydroxyl 8-qiunoline (140 mmol/l) andhydrochloric acid (200 mmol/l).
3. Buffer : ethanolamine (6 mol/l).

c. Procedure

1. Equal volumes of reagent 1 and reagent 2 were mixed wellbefore use.
2. An aliquot from each sample (20 µl) or standard wastransferred to clean tubes.
3. Portions of working reagent (1 ml) were added to sample, blankand standard tubes.
4. All tubes were well mixed and incubated for 5 min at roomtemperature.
5. The absorbance of sample and standard were measuredagainst the blank at 585 nm.

d. Calculation

Serum calcium concentration (mg/dl) = A sample / A standard × 10

Statistical Analysis

The Statistical Analysis System- SAS (2010) was used to effect of different factors in study parameters. Least significant difference –LSD test was used to significant compare between means in this study.

III. Results

Results of serum calcium lipid profile level in initial experiment in table (1)show that was not significantly in T group compare with control groupwheal results of serum calcium and lipid profile level in final experiment intable(2)show that wassignificantlyincrease($p<0.05$) in T group compare with control group wheal results of serum lipid profile levelwas decrease significantly($p<0.05$) in T group compare with control group.

Table 1. Lipid profile and calcium in initial experiment of rats.

Rat group	Serum calcium level(mg/dl)	Cholesterol (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	Triglycerides (mg/dl)
control group	8.9 ± 0.3^{a}	$79.3^{a} \pm 2.1$	$66.9^{a} \pm 1.5$	$40.2^{a} \pm 0.5$	$121.0^{a} \pm 3.5$
T group	9.2 ± 0.6^{b}	$78.5^{a} \pm 3.2$	$67.5^{a} \pm 1.2$	$41.5^{a} \pm 0.3$	$120.9^{a} \pm 2.5$

Table 2. Lipid profile and calcium in final experiment of rats.

Rat group	Serum calcium level(mg/dl)	Cholesterol (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	Triglycerides (mg/dl)
control group	9.3 ± 0.3^{a}	$80.3^{b} \pm 2.1$	$65.9^{b} \pm 1.5$	$39.5^{b} \pm 0.5$	$138.4^{b} \pm 3.5$
T group	13.2 ± 0.6^{b}	$74.5^{a} \pm 3.2$	$61.5^{a} \pm 1.2$	$37.5^{a} \pm 0.3$	$128.9^{a} \pm 2.5$

IV. Discussion

This study was based on the hypothesis that the addition oflactobacilluswill alter the intestinal development along with growth leading to changes intestinal morphology and effect on absorbed of the intestinal .

It has been reported that lactobacillus increase the calcium absorption from intestinal tract.There are more than mechanical explain the increased absorption of calcium from the intestine (Gilman et al., 2006). Fermentation products produced by Lactobacillus increase the surface area of the intestine byaccelerating proliferation in enterocytes (Scholz-Ahrens at al., 2007).Awad et al. (2010) reported that supplementations of

Lactobacillus acidophilus on intestinal morphology and cell proliferation lead to increased villus height and villus height and crypt depth ratio . Furthermore shortchained fatty acids and the other products produced by the bacteria decrease the pH ofintestines microenvironment. Therefore, calcium solubility increases and this may be relatedto increased calcium absorption (Gilman et al., 2006; Scholz-Ahrens at al., 2007).

This study has shown the effect of Lactobacillus acidophilus on intestinal absorption of cholesterol, Where it reduces the absorption of cholesterol in the intestinedue to the consumption of Lactobacillus acidophilus cholesterol for growth , the binding of cholesterolto surface of Lactobacillus, thereby inhibiting the absorption of cholesterol back into the body .the deconjugation of bile acids by bacterial acid hydrolyses, increasing cholesterol excretion of deconjugated bile salts and increasing cholesterol uptake and metabolism in the liver as compensatory response because bile acids are synthesized from cholesterol in the liver and inhibition of hepatic cholesterol and triglyceride synthesis through the action of short chain fatty acids, especially propionic acid (M. T. Liong and N. P. Shah., 2006; D. O. Noh, S. H. Kim, and S. E. Gilliland ., 1997)

References

1. M. T. Liong and N. P. Shah,(2006) "Effects of a Lactobacillus caseisynbioticon serum lipoprotein, intestinal microflora, and organicacids in rats," Journal of Dairy Science, vol. 89, no. 5, pp. 1390–1399,.
2. D. O. Noh, S. H. Kim, and S. E. Gilliland(2008),"Incorporationof cholesterol into the cellular membrane of *Lactobacillusacidophilus*ATCC 43121,"*Journal of Dairy Science*, vol. 80,n
3. Fossati, P. and Prencipe, I. (1997). Serum triglyceride determination colorimetrically with an enzyme that produce hydrogen peroxide.Clin. Chem., 28:2077-2080., pp. 3107–3113,.
4. W. T. Friedewald, R. I. Levy, and D. S. Fredrickson,(1972)"Estimation of the concentration of low-density lipoprotein cholesterol inplasma, without use of the preparative ultracentrifuge,"*ClinicalChemistry*, vol. 18, no. 6, pp. 499–502,
5. SAS,Uers Guide .(2010).Statistical ,SAS Institute Inc, Cary,NC USA.
6. Gilman, J. &Cashman, K.D. (2006).The effect of probiotic bacteria on transepithelialcalciumtransport and calcium uptake in human intestinal-like Caco-2 cells.*CurrIssuesIntestMicrobiol*, Vol. 7, No. 1, pp. (1-5).
7. Scholz-Ahrens, K. E., Ade, P., Marten, B., Weber, P., Timm, W., Açil, Y., Glüer, C.C. & Schrezenmeir, J. (2007). Prebiotics, Probiotics, and Synbiotics Affect Mineral Absorption, Bone Mineral Content, and Bone Structure.*J Nutr*, Vol. 137, pp. (838S-846S).
8. Awad, W.A., Ghareeb, K. & Bo̧hm, J. (2010). Effect of addition of a probiotic micro-organismto broiler diet on intestinal mucosal architecture and electrophysiological parameters. *J AnimPhysiolAnimNutr*, Vol. 94, No. 4, pp. (486-94).
9. S. Allender, P. Scarborough, V. Peto et al(2008)., *European CardiovascularDisease Statistics*, European Heart Network,.
10. D. J. McNamara, (2000). "Dietary cholesterol and atherosclerosis,"*BiochimicaetBiophysicaActa*, vol. 1529, no. 1-3, pp. 310–320,
11. P.-Y.Yang, Y.-C.Rui, L. Lu et al, (2005).,"Time courses of vascularendothelial growth factor and intercellular adhesion molecule-1 expressions in aortas of atherosclerotic rats,"*Life Sciences*, vol.77, no. 20, pp. 2529–2539.
12. Y.Wang, N. Xu, A. Xi, Z. Ahmed, B. Zhang, and X. Bai, "Effects of *Lactobacillus plantarum*MA2 isolated from Tibet kefir on lipid metabolism and intestinal microflora of rats fed on highcholesterol diet(2009),"*Applied Microbiology and Biotechnology*, vol. 84, no. 2, pp. 341–347,.
13. D. Gao, G. Zhu, Z. Gao, Z. Liu, L. Wang, andW. Guo(2011),"Antioxidativeandhypolipidemic effects of lactic acid bacteria frompickledchinesecabbage,"*Journal of Medicinal Plant Research*,vol. 5, no. 8, pp. 1439–1446,.
14. A. Homayouni, L. Payahoo, and A. Azizi, (2012)] "Effects of probioticson lipid profile: a review,"*American Journal of Food Technology*,vol. 7, no. 5, pp. 251–265,.
15. v FAO/WHO, *Guidelines for the Evaluation of Probiotics inFood*, Report of a Joint FAO/WHO Working Group on DraftingGuidelines for the Evaluation of Probiotics in Food, FAO/WHO, London, Canada, 2002.

16. A. Bomba, A. Brandeburov´a, J. Ri˘canyov´a et al (2012),"The role of probioticsand natural bioactive compounds in modulation of thecommon molecular pathways in pathogenesis of atherosclerosisand cancer,"*Biologia*, vol. 67, no. 1, pp. 1–13,.

*****