Iranian Biomedical Journal 4 (2 & 3): 69-73 (July 2000)

Effects of Dietary Chitosan on Serum Lipid and Lipoprotein Concentrations in Rats

Durdi Qujeq* and Gholamreza Ataei

Dept. of Biochemistry and Biophysics, Babol University of Medical Sciences, Babol, Iran

ABSTRACT

Because the ingestion of some types of dietary fibers has been shown to influence on the lipid and lipoprotein levels, it is possible that chitosan influences on lipid metabolism. In the present study, the effects of chitosan on the serum, liver lipid and lipoprotein concentrations in rat were investigated. Serum lipid level in the treatment groups were decreased compared to that of the control, cholesterol level [128.65 +/- 2.58 (mean +/- SD, n = 72) vs. 173.67 +/- 3.62, *p<0.05*] mg/dl, triglyceride level [62.83 +/- 2.73 (mean +/- SD) vs. 93.62 +/- 2.64, p<0.05] mg/dl, and low density lipoprotein-cholesterol level [108.35 +/- 2.41(mean +/- SD) vs. 156.49 +/- 2.37, *p<0.05*] mg/dl. In the chitosan treatment group, high density lipoprotein-cholesterol level was increased as compared to the control [187.39 +/- 2.74 (mean +/- SD) vs. 163.54 +/- 2.83, *p<0.05*] mg/dl. This work showed that the addition of chitosan to the diet of the rats significantly lowered the liver lipid in the treatment groups compared to that of the control, cholesterol level [31.53 +/- 1.26(mean +/- SD) vs. 64.42 +/- 2.38, *p<0.05*] mg/g, and triglyceride level [38.46 +/- 2.64 (mean +/- SD) vs. 53.24 +/- 2.45, *p<0.05*] mg/g. When chitosan fed at the 5% level, concentration of the serum cholesterol was reduced by 25.92% and triglyceride by 32.89%. The data presented here indicated possible usefulness of chitosan for the treatment of hyperlipidemia. *Iran. Biomed. J. 4 (2 & 3): 69-73, 2000*

Keywords: Chitosan, Cholesterol, Lipoprotein

INTRODUCTION

Coronary artery disease is one of the major causes of morbidity and mortality in developed countries [1]. There is a considerable body of evidence to show a strong and positive association between serum lipid, lipoprotein and this disease. A low level of serum high-density lipoprotein cholesterol (HDL-C) is considered to be a potent independent risk factor in the development of coronary heart disease [2]. An elevated plasma low-density lipoprotein cholesterol (LDL-C concentrations) is one of the major risk factors for atherosclerotic lesion [3]. The epidemiologic correlation between elevated plasma lipid levels and the incidence of atherosclerotic disease has encouraged a comprehensive search for hypolipidemic agents [4]. Intervention with both dietary or drug therapy to lower LDL-C has been shown to decrease both cardiovascular morbidity and mortality. Among the natural products, many types of non-digestible dietary fibers were found to possess hypolipidemic activity [5]. There is a considerable current interest in the hypolipidemic activities of dietary non-nutritive substance. Chitosan is one of the known substances to affect the levels of lipids in serum and liver. It is a polymer containing glucosamine units that may cause improvement of the fatty liver and hyperlipidaemia in mice fed a high fat diet through inhibiting intestinal absorption of the dietary fat [6]. Chitosan is a polycationic polymer obtained commercially by alkaline deacetylation of chitin (a N-acetyl-glucosamine polymer) from shellfish exoskeletons. Chitosan is inexpensive and non-toxic and possesses potentially reactive amino functional groups. It has been shown to have a potential use in many different fields, including as an antifungal compound in agriculture, flocculating agent in wastewater treatment, a food additive in alimentary industries, hydrating agent in cosmetics

* Corresponding Author. Abbreviations: LDL-C, Low density lipoprotein-cholesterol; HDL-C, High density lipoprotein-cholesterol.

[Downloaded from ibj.pasteur.ac.ir on 2024-03-28]

[DOR: 20.1001.1.1028852.2000.4.2.3.2]

and, more recently, in biomedicine and several pharmaceutical preparations [7, 8]. The addition of chitosan to diet decreased the apparent fat absorption ratio and abdominal fat pad weight in chickens fed on the basal and high fat diets. The increased plasma triglyceride concentration due to feeding the high fat diet was decreased by addition of chitosan to the diet [9]. Chitosan-containing diets generally reduce total plasma cholesterol and HDL-C concentration [10]. Chitosan effectively lowers the cholesterol absorption more than guar gum or cellulose did, and this effect was more significant when given with fiber. Dietary fats did not modify cholesterol absorption [11]. The hypoglycemic and hypolipidemic effects of chitosan, a polymer of glucosamine, were investigated in neonatal streptozotocin diabetic mice. Chitosan reduced blood glucose, cholesterol and triglyceride of neonatal streptozotocin diabetic mice [12]. Chitosan feeding affects the metabolism of intestinal bile acids in rats [13]. Addition of chitosan to the diet did not affect the body gain weight and feed efficiency [14]. Previous studies have low examined the influence of chitosan on serum lipoprotein levels. In view of these considerations, the effects of the chitosan on the serum and liver cholesterol, triglyceride and lipoprotein levels in rats were investigated.

MATERIALS AND METHODS

Chitosan, lactic acid, Cibacron brilliant red 3B-A powder, $MgCl_2$, phosphotungstic acid, cholesterol oxidase, peroxidase, 4-aminophenazone and polyvinyl sulfate were obtained from Sigma St. Louis, Mo., USA. All of the chemicals used were guaranteed grade reagents. All solutions were prepared with distilled and deionised water.

Animals. Male Sprague-Dawley rats weighing approximately 220-240 g, 6-8 weeks old, were used in these studies. All animals survived the study without signs of illness. All experiment manipulations were carried out with the animals under ether inhalation anesthesia. The animals were maintained in an air-conditioned room at 19-23°C, with a 12-h light-dark cycle, and acclimated for 3-4 days before starting the experiments.

Body weight and food consumption were recorded every day. Blood samples were obtained at least every 12 h for measurement of serum lipids and lipoproteins. The animals were divided into the following two groups:

Group 1 (control). Animals were fed with a control diet consisting of a commercial powder diet. The composition of the basal diet was (%): Casein, 25; lard, 15; mineral mixture, 5; vitamine mixture, 2; cellulose powder, 2; choline chloride, 0.2; and sucrose to 100.

Group 2 (treatment). Animals were fed with a diet supplemented with 5% chitosan. Except for the ingestion of chitosan, they were given the same diet as the rats in the control group.

The animals were maintained on these diets at libitum for 28 days. Food intake was measured daily, and body weight was determined prior to killing. Finally, animals were sacrificed by cardiac puncture under diethyl ether anesthesia, after overnight fasting. The blood was collected, the serum was separated from the blood. The liver was excised, at least four to six livers from four to six rats were pooled as the tissue size was small (50 mg), homogenized in 5.0 ml of 0.1 M Tris-HCl buffer (pH 7.8), and centrifuged at 12,000 g at 4°C in a refrigerated high-speed centrifuge (Sorval RC 5B). The supernatant was used as the lipid extract.

Test sample. Tablets containing chitosan with an average molecular weight of approximately 300,000 Da and a degree of deacetylation of 95% were administered orally to the test subjects. A calorimetric procedure was used for the determination of chitosan content. The oven-dry chitosan powder (1 g) was suspended in distilled and deionised water (5 ml), after stirring for 20 min, lactic acid (96%. 0.2 ml) was added to dissolve chitosan. A solution of the dye was prepared by dissolving the Cibacron brilliant red 3B-A powder (10 mg) in distilled and deionised water (2 ml). Chitosan solution, 0.7 ml was introduced into test tubes, followed by 0.3 ml of dye solution to reach one ml. The absorbance values were measured at 570 nm with Cecil CE spectrophotometer [15].

Isolation of LDL and HDL. Lipoproteins were isolated by sequential ultracentrifugation of 0.5 ml of serum on Beckman L8-M ultracentrifuge at 120,000 ×g for 24 h. The lipoproteins were identified at the following densities: between 1.030 and 1.050 kg/l for LDL and between 1.063 and 1.21 kg/l for HDL.

HDL-C assay. In this assay, 0.5 ml of serum was mixed with 0.5 ml of solution containing 0.2

[Downloaded from ibj.pasteur.ac.ir on 2024-03-28]

[DOR: 20.1001.1.1028852.2000.4.2.3.2]

mmol/l phosphotungstic acid (PTA) and 5 mmol/l $MgCl_2$. After a 5-min incubation and precipitation at room temperature, samples were centrifuged at 5,000 ×g for 15 min. The supernatant was removed manually for assays, and then HDL-C was determined with an enzymatic end point assay, by using cholesterol oxidase and peroxidase and then a chromogenic reaction with 4-aminophenazone (CHOD-PAP) on a Cecil CE spectrophotometer [16].

LDL-C assay. For this analysis, we used the polyvinyl sulfate method. Briefly, a mixture of 0.5 ml of serum and 0.2 ml of reagent containing polyvinyl sulfate was incubated for 10 min at room temperature, and then centrifuged for 20 min at 2,000 ×g. After centrifugation, the cholesterol content of the supernatant was determined by the CHOD-PAP method. The LDL-C concentration was calculated by the difference between the total serum cholesterol and the cholesterol in the supernatant [17].

Lipid measurements. Concentration of cholesterol and triglycerides were enzymatically determined with the (CHOD-PAP) and glycerophosphate oxidase-proxidase-4-amino-phena-zone (GPO-PAP) methods, respectively, on a Cecil CE spectrophotometer. The interassay CV for determinations of total cholesterol and total triglycerides varied between 1.23% and 2.41% and between 1.87% and 3.46%, respectively [18].

Statistical analysis. The significance of differences between the mean of the control and test groups was determined by one-way analysis of variance followed by student's *t-test*. The $p<0.05$ was considered to indicate statistical significance.

RESULTS

The results of food intake, gain weight and liver weight were shown in Figure 1. Gain weight was significantly lower than control group, although food intake was similar. The liver weight was significantly lower in chitosan group than in control group. The results of liver lipid analyses were shown in Figure 2. Liver cholesterol concentrations decreased remarkebly, 51.06% in treatment group. Figure 3 shows that chitosan was extremely effective in preventing the rise of serum triglyceride, 32.89% and cholesterol, 25.92% as compared to that of control group. The triglyceride values tended to shift to a slightly lower level in the treatment group throughout the test period.

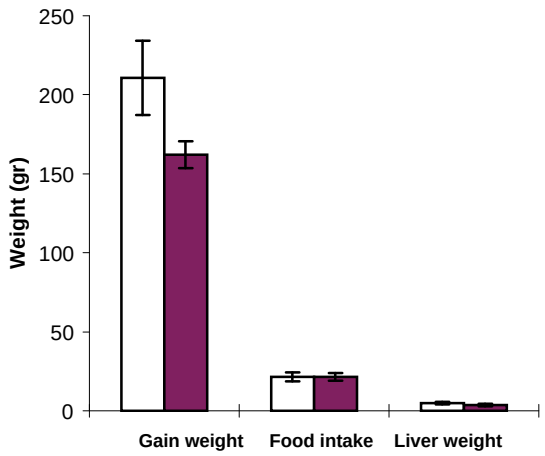


Fig. 1. Effect of chitosan on gain weight, food intake and liver weight in control (1, light) and treatment group (2, dark). Feeding period is 28 days. Each column represents the mean value +/- SD of 6 independent experiments.

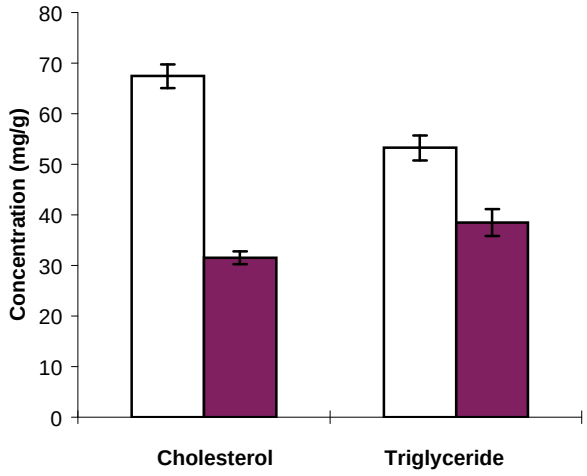


Fig. 2. Effect of chitosan on levels of cholesterol and triglyceride in liver. Control group (white) and treatment group (dark). Feeding period is 28 days. Each column represents the mean value +/- SD of 6 independent experiments.

[Downloaded from ibj.pasteur.ac.ir on 2024-03-28]

[DOR: 20.1001.1.1028852.2000.4.2.3.2]

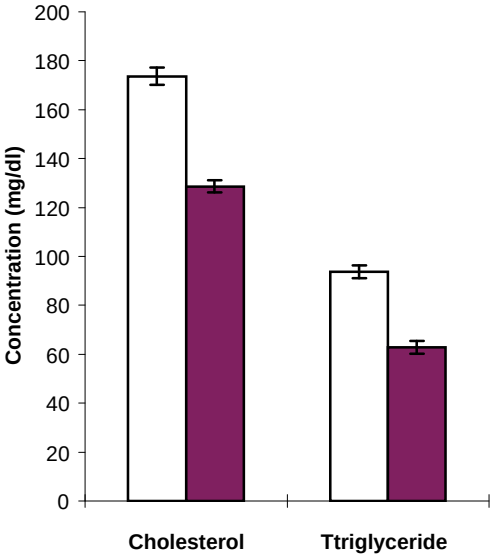


Fig. 3. Effects of chitosan on levels of cholesterol and triglyceride in serum. Control group (white) and treatment group (dark). Feeding period is 28 days. Each column represents the mean value +/- SD of 6 independent experiments.

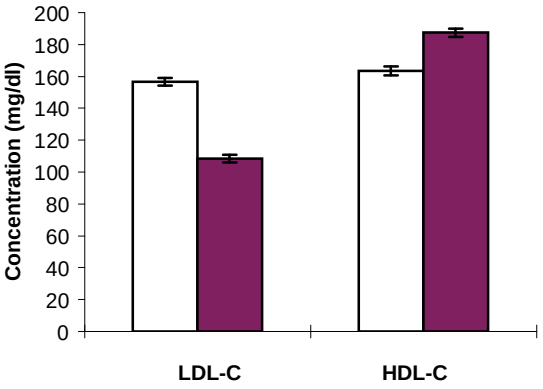


Fig. 4. Effect of chitosan on levels of LDL-C and HDL-C in serum. Control group (white) and treatment group (dark). Feeding period is 28 days Each column represents the mean value +/- SD of 6 independent experiments.

After ingestion of chitosan for 28 days, the average LDL-C level of the rat in the treatment group decreased significantly, 30.76%. A significant increase in average serum HDL-C level was also observed after 28 days of chitosan ingestion by the rats in the treatment group (Fig. 4).

DISCUSSION

According to the data obtained, food intake was not apparently influenced by dietary chitosan, but gain weight and liver weights tended to decrease (Fig. 1). Our results are in good agreement with results obtained previously [9, 10]. Results of this study like other investigators [10, 11] suggest that the reductions of liver cholesterol and triglyceride in rats (Fig. 2) indicate that chitosan effectively interferes with cholesterol absorption. Chitosan feeding induced a significant reduction of plasma cholesterol in mice fed a hyper-cholesterolemic diet consistent with previous reports [5, 6]. The present study also shows that chitosan feeding (5%) reduces serum cholesterol and triglyceride levels in rats (Fig. 3). Results obtained from this paper, like those of other authors [11, 12] suggest that chitosan has demonstrable lipid lowering activity in rats. According to these reports, it is clear that chitosan combines with bile acid in the digestive tract and that the product is excreted in the faeces, thereby reducing the body's lipid level.

Serum HDL-C level was increased significantly by the chitosan feeding in comparison with control feeding. Also, serum LDL-C level was decreased by the chitosan feeding, which was attributed to the general reductions in serum cholesterol concentrations, probably caused by enhanced reverse cholesterol transport in response to intestinal losses of dietary fats (Fig. 4). These results confirm findings of other investigators [13, 19].

Thus, chitosan may affect cholesterol absorption both by binding cholesterol and presumably by disrupting micelle formation in the intestine. Consequently, chitosan might cause improvement of the fatty liver and hyperlipidaemia in rats through inhibiting intestinal absorption of dietary fat. Therefore, it is likely cholesterol that was not absorbed by the proximal region of the small intestine, the site of cholesterol absorption, accumulated in the cecum which may explain decreased serum and liver cholesterol levels. In our study, chitosan was clearly found to be considered the most powerful hypolipidemic natural product discovered so far. Because of these considerations together with its low toxicity, chitosan seems to be a realistic hypocholesterolemic agent. Possibly, the general hypolipidemic action of chitosan will make this polycationic polymer the new important target of nutritional and biochemical studies. This article highlights the importance of this novel material for

[Downloaded from ibj.pasteur.ac.ir on 2024-03-28]

[DOR: 20.1001.1.1028852.2000.4.2.3.2]

a variety of biomedical applications. Further, this paper provides an insight to the further perspectives for using it in clinical practice.

REFERENCES

1. Troyler, H.A. (1984) Cholesterol and cardiovascular diseases. *Am. J. Cardiol. 54: 14C-19C*.
2. Miller, G.J. and Miller, N.E. (1975) Plasma high-density lipoprotein concentration and development ischemic heart disease. *Lancet I: 16-17*.
3. Caslake, M.J., Packard, C.J., Gaw, A., Murray, E., Griffin, B.A.,Vallance, B.D. and Shepherd, J. (1993) Fenofibrate and LDL metabolic heterogeneity in hypercholesterolemia. *Arterioscler. and Thromb. 13: 702-711*.
4. Trowell, H. (1972) Ischemic heart disease and dietary fiber. *Am. J. Clin. Nutr. 25: 926-929.*
5. Miettinen, T.A. and Tarpila, S. (1977) Effect of pectin on serum cholesterol, fecal bile acids and billiary lipids in normolipidemic and hyperlipidemic individuals. *Clin. Chim. Acta. 79: 471-477.*
6. Han, L.K., Kimura, Y. and Okauda, H. (1999) Reduction in fat storage during chitin chitosan treatment in mice fed a high fat diet. *Int. J. Obes. Relat. Metab. Disord. 23: 174-179.*
7. Akbuga, J. (1993) Use of chitosonium malate as a matrix in sustained release tablets. *Int. J. Pharm. 89: 19-24.*
8. Pelletier, A., Lemire, I., Sygusch, J., Chornet, E. and Overend, R.P. (1990) Chitin/chitosan transformation by thermo merchano-chemical treatment including characterization by enzymatic depolymerization. *Biotechnol. Bioengin. 36: 310-315.*
9. Jing, S. and Yamaguchi, T. (1992) Removal of phosphate from dilute phosphate solution by an iron chitosan complex to be used as an oral sorbent. *Bull. Chem. Soc. Jpn. 65: 1866-1870*.
10. Razdan, A. and Pettersson, D. (1994) Effect of chitin and chitosan on nutrient digestibility and plasma lipid concentrations in broiler chickens. *Br. J. Nutr. 72: 277-288*.
11. Ikeda, I., Tomari, Y. and Sugano, M. (1989) Interrelated effects of dietary fiber and fat on lymphatic cholesterol and triglyceride absorption in rats. *J. Nutr. 119: 1383-1387*.
12. Miura, T., Usami, M., Tsuura, Y., Ishida, H. and Seino, Y. (1995) Hypoglycemic and hypolipidemic effect of chitosan in normal and neonatal streptozotocin induced diabetic mice. *Biol. Pharm. Bull. 18:1623-1625*.
13. Fukada, Y., Kimura, K. and Ayaki, Y. (1991) Effect of chitosan feeding on intestinal bile acid metabolism in rats. *Lipids 26: 395-399*.
14. Maezaki, Y., Tsuji, K., Nakagawa, Y., Kawai, Y., Akimoto, M., Tsugita, T., Takekawa, W., Tarada, A., Hara, T. and Mitstuoka, T. (1993) Hypocholesterolemic effect of chitosan in adult males. *Biosci. Biotech. Biochem. 57: 1439-1444*.
15. Muzzarelli, R.A. (1998) Colorimetric determination of chitosan. *Anal. Biochem. 260: 255-257*.
16. Lopes-Virella, M.F., Stone, P., Ellis, S. and Colwell, J.A. (1977) Cholesterol determination in high-density lipoproteins separated by three different methods. *Clin. Chem. 23: 882-884*.
17. Assmann, G., Jabs, H.U., Kohnert, U., Nolte, W. and Schriewer, H. (1984) LDL-cholesterol determination in blood serum following precipitation of LDL with polyvinyl sulfate. *Clin. Chim. Acta. 140: 77-83*.
18. Maitra, A., Hirany, V. and Jialal, I. (1997) Comparison of two assays for measuring LDL cholesterol. *Clin. Chem. 43: 1040-1047*.
19. Razdan, A. and Pettersson, D. (1996) Hypolipidaemic, gastrointestinal and related responses of broiler chickens to chitosans of different viscosity. *Br. J. Nutr.76: 387-397*.

[Downloaded from ibj.pasteur.ac.ir on 2024-03-28]

[DOR: 20.1001.1.1028852.2000.4.2.3.2]