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Effect of Prebiotics Inulin and Mannan on Lipid Profile and Intestinal Micro Flora of Hypercholesterolemic Rats

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ABSTRACT

The current study was aimed to determine the effect of Inulin and mannan on the lipid profile and possible reduction of serum cholesterol level of rats induced hypercholesterolemia. Twenty-eight male Wistar rats were divided into four experimental groups as follow; control group: fed standard commercial diet; HC: standard diet containing 1% cholesterol and 10% egg yolk powder; HC+M: HC+ 5% mannan; HC+I: HC+ 5% inulin. During 50 days, serum lipid profile, serum ALT and AST, liver total cholesterol and triglyceride, the body and organ weights were measured. HC+M and HC+I groups showed significantly lower cholesterol, LDL–cholesterol, atherogenic index and serum AST than HC group and also liver triglyceride and total cholesterol were significantly less in HC+M and HC+I. HC+M and HC+I diet had significantly controlled rats weight gain. These data demonstrate that mannan and inulin may be useful and can be used as an alternative or complementary treatment in hypercholesterolemia and related disease conditions.

KEY WORDS: Hypercholesterolemia; Inulin; Mannan; Lipid profile

INTRODUCTION

Cardiovascular diseases were considered as the major causes of adult's death, worldwide. It has been reported that elevation of certain blood lipids levels are principal cause of cardiovascular disease and coronary heart diseases [1]. Diets rich in saturated fat and cholesterol and poor in fiber, may result in elevated plasma cholesterol levels [2].

Despite the long introduction of the dietary fiber in 1953, the health benefits of them were repeatedly addressed [3]. Prebiotics, including dietary fibers and fructo-oligosaccharides, are inedible beneficially food elements which improving the host health through the enhancement of growth and activity of certain micro-flora of the host gastro-intestinal tract[4, 5].

Prebiotics have an important role in diets because of several functions, including: allowing growth and balancing of gut micro biota, altering the composition of the micro flora of gastrointestinal tracts, acting as alternative to probiotics, preventing some disease such as diarrhea, hypercholesterolemia and hypertension and increasing immune system of the body [4].

Inulin and mannan are classified as insoluble dietary fibers. Inulin is formed by the composition of several chains of fructose units linked by β (2–1) glycosidic bonds frequently ended by a glucose unit. Mannan-oligosaccharides are naturally present in the cell walls of the yeast *Saccharomyces cerevisiae* and can be obtained by centrifugation of lysed yeast culture [6, 7].

It was formerly hypothesized that increase in the formation of such fermented byproducts can potentially reduce the synthesis of hepatic cholesterol, moreover, a disruption in the circulation of bile acids in the entero-hepatic system and also fecal emission of the extra bile salts are among the major outcomes of hypocholesterolemia [8, 9].

to explore the effect of feeding two kinds of prebiotic (inulin and mannan) on the lipid profile and possible reduction of serum cholesterol level of rats induced hypercholesterolemia by adding high dose of cholesterol and egg yolk powder to their diet and to study their improving intestinal micro flora by these prebiotics.

MATERIALS AND METHODS

Experimental animals

Twenty eight male Wistar rats (200±10 gram) were randomly divided into four groups (n=7). The animals were maintained in a central, small animal house under a controlled temperature at 22±2°C and relative humidity of 56% to 60% with 12-h light exposure in a daily cycle from 7 a.m. to 7 p.m. and were fed on a commercially diet (The

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standard pellet feedstuff contained 17.9% protein, 48.5% starch, 4.9% sugar, 5.3% crude fiber, 4.6% fat and 7.1% ash, 0.72% calcium, 0.6% phosphorus, 0.23% magnesium and 0.25% chloride). Animals were fed *ad libitum*. All animals were adapted for a period of one week before the experimental session.The individual rat weight was recorded weekly throughout the experiment.

All over the experimental procedures, the ethics of animal welfare was approved by Shiraz University Animal Welfare Laws, Guidelines and Policies in Iran).

Diet and experimental design

The four experimental group included a control: normal diet (commercial diet); HC: high-cholesterol diet (commercial diet with 1% cholesterol (Merck, Darmstadt, Germany) and 10% egg yolk powder (Narin company, Iran)); HC+I: high-cholesterol diet+5% inulin (Sensus, Netherland); HC+M: high-cholesterol diet+5% mannan (Soren tech, Iran).

After one week of adaptation the animals were fed *ad libitum* during 50 days and body weight were recorded every week.

Microbial analysis of fecal samples

In order to the microbial analysis of the animals, fecal samples were separately obtained during 21 and 50days following onset of the trials which was promptly processed within 1 h post collection. The samples were subsequently homogenized using a stomacher. The preparations were then serially diluted followed by subculture in triplicate on plate count agar (Merck, Darmstadt, Germany)for total count and VRBD agar (Merck, Darmstadt, Germany) for Enterobacteriaceae count, and incubated aerobically at 37°C for 48 h.

Oral glucose tolerance test

After the dietary administration, at days 28 and 50, four and three animals were respectively deprived of the diets for 12 h in order to take glucose solution (5g/kg) by intra gastric gavage. The oral glucose tolerance test was then performed on the blood samples were taken from tail vale. The blood glucose was recorded at 0, 30, 60, 90, and 120 min using a rapid blood glucose meter (Accu-chek company, USA)[10].

Samples collection

At the end of days 28 and 50 of the trials, the overnight-fasted rats were anesthetized by diethyl ether, the serum samples were then collected from heart following centrifugation at 3000×g for 15 minutes and kept at -70°C for further analysis.

Liver, heart, spleen and kidneys were removed, rinsed in chilled saline solution, blotted on filter paper, and weighed separately. The organs were kept at -20°C until analysis. The analysis of liver for determination of total cholesterol and triacylglycerols was finally carried out.

Serum and liver lipids analytical procedures

Total cholesterol (TC), triglyceride (TG), and high-density lipoprotein (HDL) cholesterol of the sera were analyzed by colorimetric reactions using specific enzymatic kits (Biorex Fars, Iran).To analyze other plasma lipid fractions including Low-density lipoprotein (LDL) cholesterol, the Friedewald equation was employed to calculate the very low-density lipoprotein (VLDL) cholesterol, Atherogenic index (AI), LDL/HDL ratio and TC/HDL ratio [11].

Liver lipids were extracted and purified according to the method of Hara, et al. (1978) with few modifications [12]. Briefly, 18 ml of hexane: isopropanol (3:2) was added to 1 gram of liver sample, the mixture was homogenized(DI 18B, IKA, Germany) at 5000×gfor 30s and maintained over night at 4°C. The mixture was centrifuged at 5000×g for 20 min. Non-lipid constituents in the extract were removed by mixing two time volumes of the supernatant with 12 ml of aqueous sodium sulfate (prepared from 1g of the anhydrous salt and 15 ml of water). The hexane-rich layer was carefully collected the distinct upper aqueous phase. The extracted TG and cholesterol were measured using commercial enzymatic kits (Biorex Fars, Iran).

Serum enzymatic analytical procedures

The serum samples were analyzed for liver enzymes ALT and AST by colorimetric reactions using specific enzymatic kits (Biorex Fars, Iran).

Statistical analysis

The data of every three replicates were analyzed using SPSS (version 19.0) software and subjected to one-way analysis variance. The difference of means between groups was also analyzed using Duncan's multiple comparison test. The level of $P < 0.05$ was considered as significant.

RESULTS AND DISCUSION

In the lipid-lowering potential of dietary inulin and mannan in Wistar rats was studied, here. There is a lack of information to compare the effect of inulin and mannan in lipid profile of hypercholesterolemic rats. Mannan and inulin are considered as dietary fiber. The dietary fibers are likely fermentable by fecal bacteria of the cecum and colon to produce short-chain fatty acids (acetate, propionate and butyrate) and lactate [13].

The effects of administering dietary fibers on serum lipid profile (serum total triglyceride, cholesterol, HDL-cholesterol, LDL-cholesterol, VLDL-cholesterol, atherogenic index, HDL/LDL cholesterol ratio and cholesterol/ HDL ratio) at the days 28 and day 50 post exposure are shown in Fig.1.

The statistical analysis of serum triglycerides (Fig. 1A) showed no significance difference between treatment groups. However serum triglycerides was significantly higher at day 50, than day 28 ($P<0.05$).

On day 28, treatment groups receiving mannan (HC+M) and inulin (HC+I) showed significantly lower cholesterol than HC group such that a decrease of serum cholesterol by 33.9% and 21.1%, respectively was observed. On day 50, serum cholesterol in HC group was 30.1% higher than control group ($P<0.05$) and HC+M diet significantly controlled serum cholesterol and was similar to control diet. Inulin showed observable effect on serum cholesterol, but this effect did not appear to be significant (Fig. 1B). These results showed that mannan had more controlling effect than inulin on days 28 and day 50 of treatment.

A significant postprandial triglyceride lowering effect (27% to 61%) subsequent to the oral administration of 10 g oligofructose /100g diet to male rats fed high-fat diet was reported by Delzenne and Kok (1999). The effect was likely caused by reducing the activity and expression of all lipogenic enzymes in the liver [14]. The hypothesis was later more clarified when it was described that the main influences are resulted from reduction in the hepatic de novo fatty acid and triacylglycerol synthesis [8]. Gallaher et al. (2000) also showed significantly reduction of serum and liver cholesterol of rats fed 10% glucomannan and chitosan. They suggested that increasing the viscosity of intestinal contents caused by glucomannan feeding was potentially associated with lowering of cholesterol absorption [13].

In this study HC diet caused 51.9% and 137.4% increase in serum LDL-cholesterol after 28 and 50 days of treatment ($P<0.05$). HC+M and HC+I groups showed significant decreasing effect on LDL–cholesterol on days 28 and 50 ($P<0.05$). Decreased LDL-cholesterol on day 28 for HC+M and HC+I groups were 65.3 and 59.0%, respectively compared to control ($P<0.05$). On day 50 prebiotic diets significantly controlled LDL-cholesterol which was similar to control diet. Our results also showed no significant difference for cholesterol lowering effect of mannan and inulin diet ($P>0.05$) (Fig. 1C).

No significant difference in the serum HDL-Cholesterol was shown between the experimental groups on the days 28 and 50, except for HC+I diet which showed significant increase by 19.5% compared to control group on day 28. Despite of having high cholesterol diets, rats had higher serum HDL-cholesterol on day 50 than day 28 (Fig. 1D).

Statistical analysis of serum VLDL-cholesterol showed no significant difference between treated groups on both day 28 and day 50 (Fig.1E). It has been hypothesized that the principal mechanism for the lipid lowering action of prebiotics was resulted from changes in the hepatic de novo lipogenesis, especially those affecting VLDL production and secretion, the changes were prebiotic dose dependent [15].

On day 28 of treatment atherogenic index was significantly lower in rats fed HC+M and HC+I diet by 42.4 and 46.6%, respectively compared with controls, whereas it was significantly greater in rats fed HC diet. There was no significant lowering effect between HC+M and HC+I diet on day 28 but HC+M had significantly more lowering effect of atherogenic index on day 50 (Fig.1F).

Results of serum Cholesterol/HDL ratio are shown in Fig.1G which showed significant decrease for both HC+M and HC+ I diet on day 28. HC+I diet also showed significant decreasing effect on day 50.

On the 28^{th}daysof the experiments a significant changes in the serum LDL/HDL cholesterol was observed among all the trials. HC diet significantly increased LDL/HDL cholesterol ratio by 56.8% compared to control whereas HC+M and HC+I diet was significantly decreased this ratio by 64.9% and 64.7% and there were no significant different between HC+M and HC+I diet. On day 50 also HC+M and HC+I diet significantly decreased LDL/HDL cholesterol ratio compared to HC group (Fig.1H).

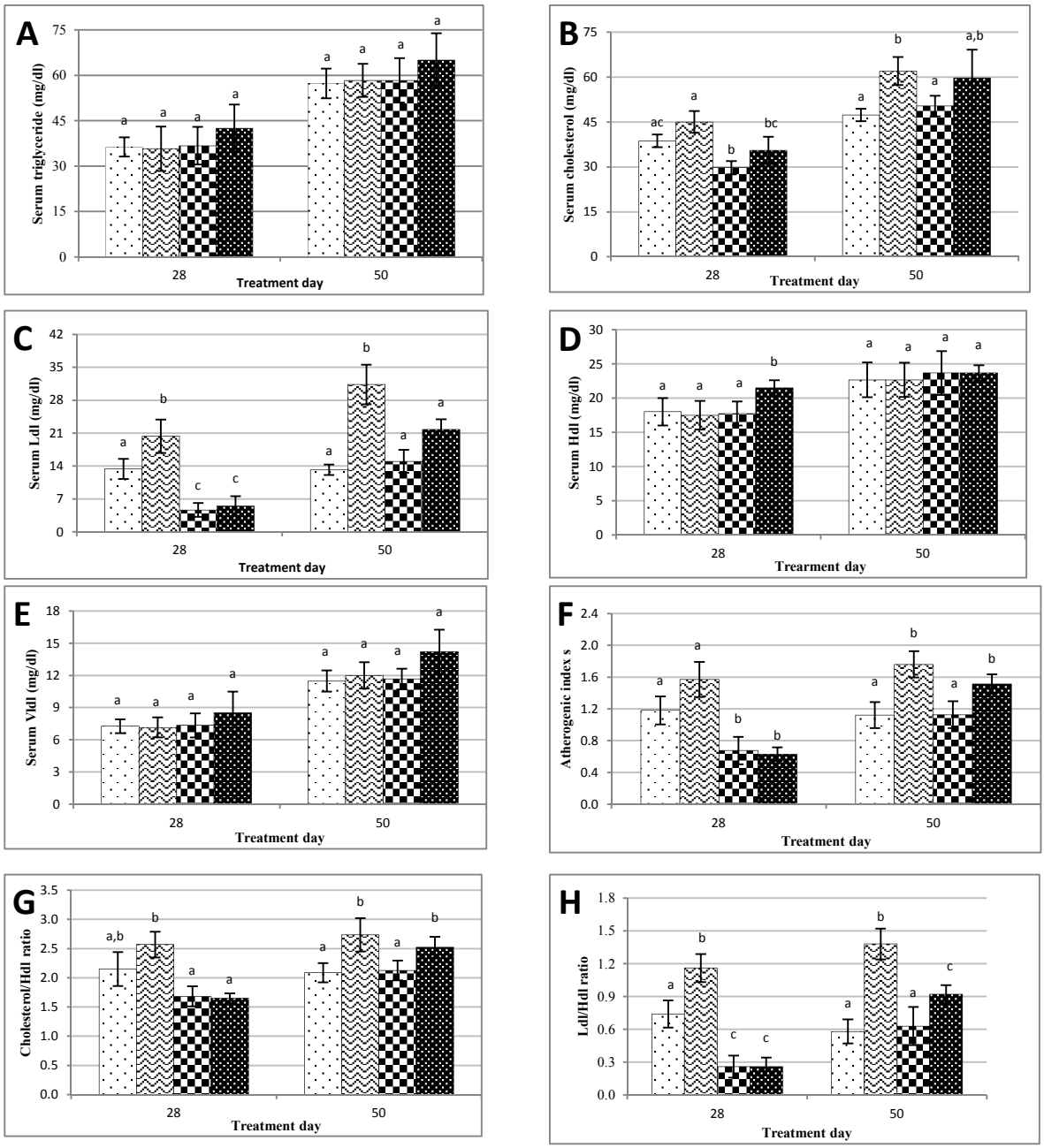


Fig. 1. The effect of supplementation of diet with prebiotics mannan and inulin on A: serum triglyceride, B: total cholesterol, C: LDL- cholesterol, D: HDL-cholesterol, E: VLDL-cholesterol, F: atherogenic index, G: Cholesterol/ HDL ratio and H: LDL/HDL ratio in hypercholesterolemicmale Wistar rats. Control ( ), High cholesterol ( ), High cholesterol + mannan ( ), High cholesterol + inulin ( ). Bars represent standard deviation values. Different letters in the same sampling day indicate significant differences at the level of 0.05.

The activities of ALT and AST were measured to access hepatocellular damage [16]. Serum ALT and AST are shown in the Fig.2. No significant difference in serum ALT activity of all groups was seen on day 28 but it significantly decreased in rats fed HC+I diet on day 50 by 38.8% compared to HC diet and was close to control group (Fig.2A).HC diet also had a significant increasing effect on serum AST on days 28 and 50 by 20.19% and 16.38% respectively, which serum AST in mannan and inulin group was similar to control group on day 28 and even significantly less in day 50 (Fig.2B).These observations led to the conclusion that high cholesterol diet can induce hepatic damage resulting in elevated hepatic enzymes and that prebiotics can alleviate these changes. These results

were supported by El-Mahmoudy et al.(2014)who investigated the effect of mannan-oligosaccharide on the lipogram in hyperlipidemic rats [16].

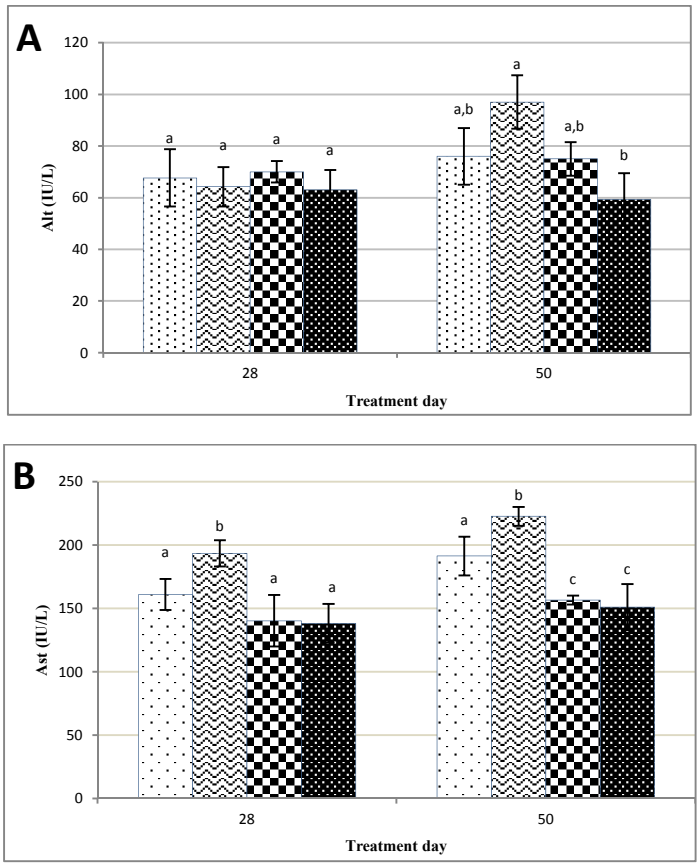


Fig. 2. The effect of supplementation of diet with prebiotics mannan and inulin on A: serum ALT and B: serum AST in hypercholesterolemicmale Wistar rats. Control ( ), High cholesterol ( ), High cholesterol + mannan , High cholesterol + inulin ( ). Bars represent standard deviation values. Different letters in the same sampling day indicate significant differences at the level of 0.05.

HC diet increased liver triglyceride significantly ($P<0.05$). Statistical analysis for the effect of feeding different prebiotic on liver total triglyceride revealed that there was a higher significant decreasing effect on day 28 and 50 by 21.5% and 31.1% for HC+M diet and 20.9% and 30.1% for HC+I diet respectively than did the corresponding HC diet ($P<0.05$) (Fig. 3A).

HC diet increased liver total cholesterol significantly($P<0.05$). Effect of dietary mannan and inulin on liver total cholesterol showed the significant decrease on days 28 and 50 compared to HC and also control group (Fig. 3B).

Our results were also supported by Delzenne et al. (1999) which explained dietary supplementation with oligofructose (100g/kg), decreases serum triacylglycerols in serum and VLDL of rats. It also showed significantly decrease in triacylglycerol and phospholipid concentrations in both blood and liver [14]. Gallaher (2000) also showed significantly reduction of total liver cholesterol by adding 10% of glucomannan and chitosan tohypercholesterolemic rats [13].

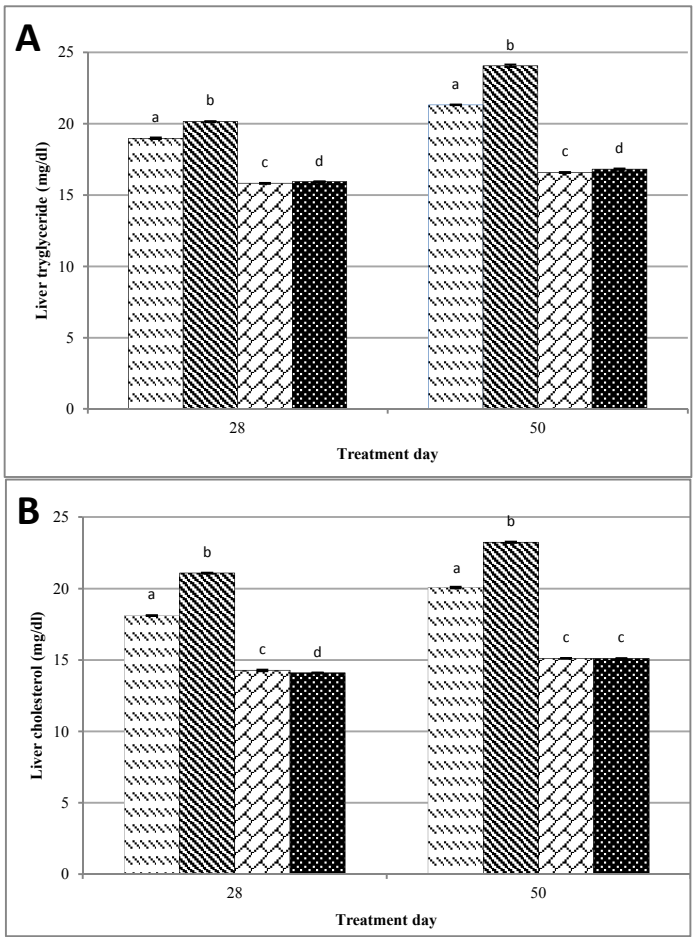


Fig. 3. The effect of supplementation of diet with prebiotics mannan and inulin on A: liver triacylglyceride and B: liver total cholesterol in hypercholesterolemicmale Wistar rats. Control (▨), High cholesterol (▨), High cholesterol + mannan (▨), High cholesterol + inulin (▨). Bars represent standard deviation values. Different letters in the same sampling day indicate significant differences at the level of 0.05.

The effects of the dietary treatments on the fecal total count and Enterobacteriaceae count are presented in Fig. 4. After 21 and 50 days of treatment, fecal total count of HC group was significantly lower compared to the other groups ($P<0.05$). However, total count in HC+M and HC+I groups were similar or even more than control group (Fig.4A). It is possible that high concentration of cholesterol in the diet of HC group has prevented growth of some species of bacteria in the large intestine of rats. However, in the HC+M and HC+I groups; it appears that the stimulating effect of prebiotics on bacterial growth has indeed overcome the inhibitory influence of cholesterol.

HC, HC+I and HC+M diets significantly increased fecal Enterobacteriaceae count compared to control diet after 21 days ($P<0.05$). However, no significant changes were observed between all the groups over the 50 days (Fig. 4B).Our results support the concept of selective stimulation of feces bacteria by prebiotics [4, 5, 17].

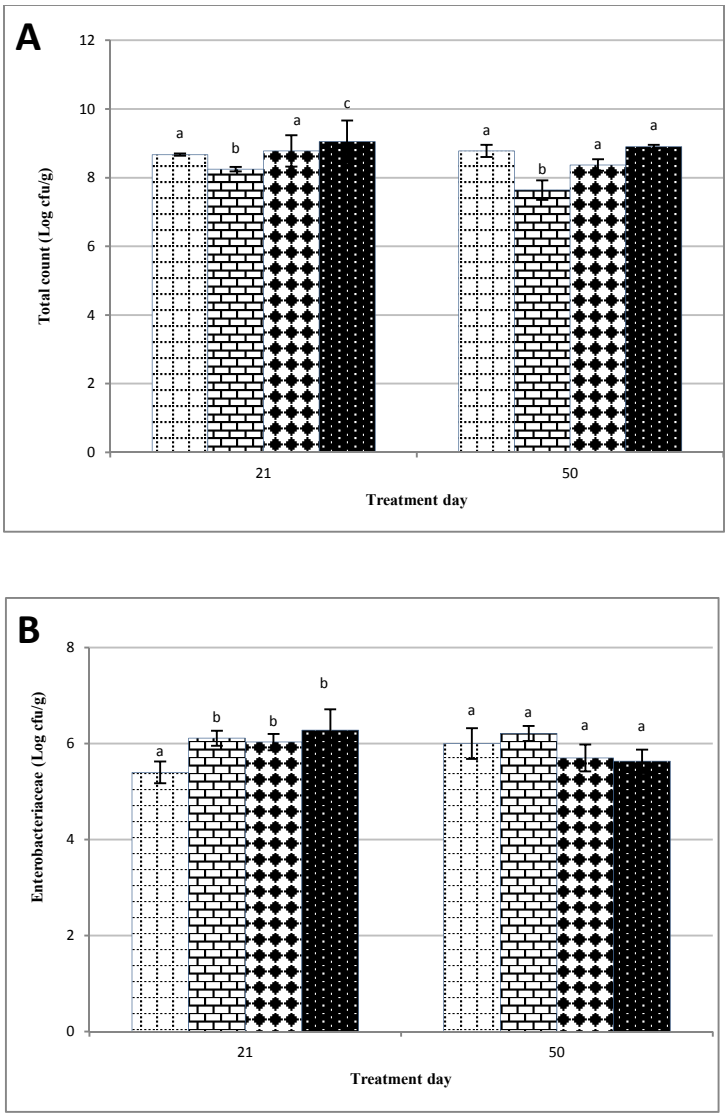


Fig. 4. The effect of supplementation of diet with prebiotics mannan and inulin on A: Total count and B: Enterobacteriaceae count of feces in hypercholesterolemicmale Wistar rats. Control ( ), High cholesterol ( ), High cholesterol + mannan ( ), High cholesterol + inulin ( ). Bars represent standard deviation values. Different letters in the same sampling day indicate significant differences at the level of 0.05.

Weight gain for HC group was significantly more on days 35, 42 and 50 and HC+M and HC+I diet had significantly controlled their weight gain compare to HC diet(Fig.5). The weight gains during the study period were respectively 121.0g and 144.4g in the control and the HC diet groups. However, when feeding the HC+M and HC+I diet, the weight gain was 95.5g and 105.0g, respectively. Gallaher et al. (2000) showed weight gain reduction of rats when fed 10% glucomannan and chitosan compared to control diet and explained that the lower daily food intake of these groups are the reason of weight control [13].A relative lower weight gain in the prebiotic ingested group was probably associated with the presence of large amounts of the insoluble fibers in the GIT. This phenomenon was previously reported by earlier studies that diets containing high insoluble fibers producing less metabolize energy [18].

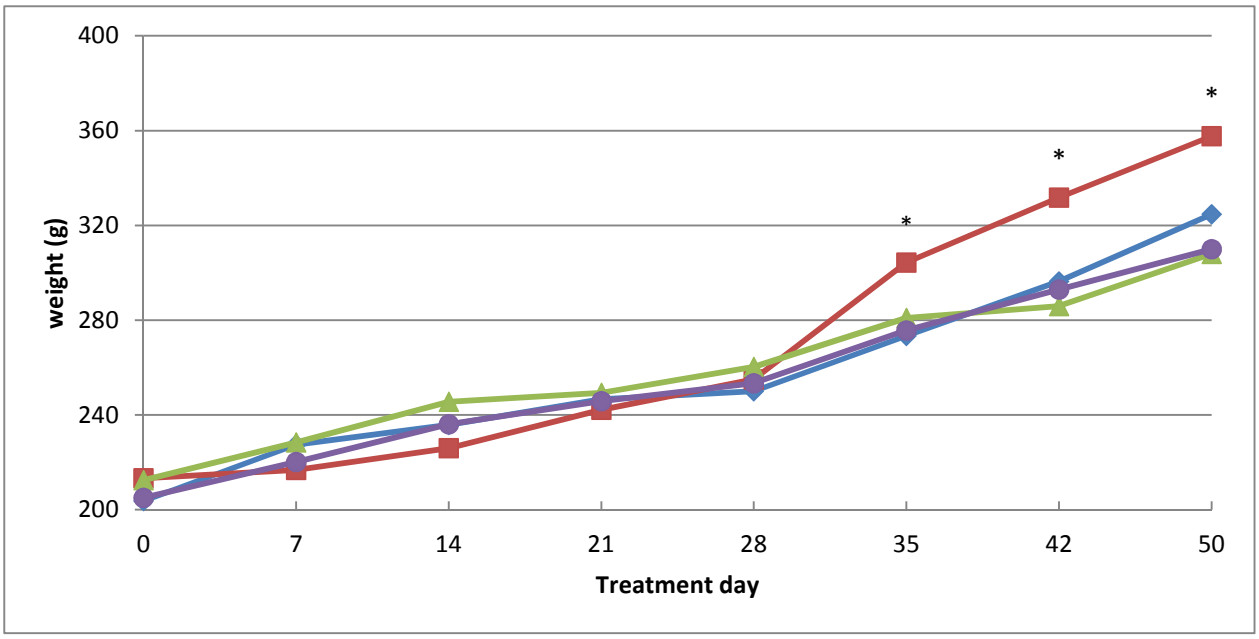
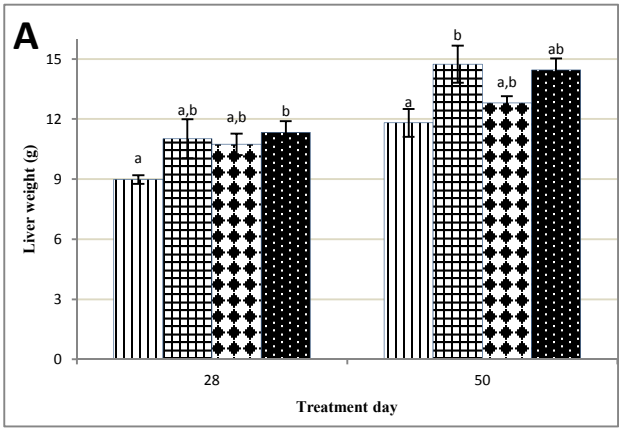
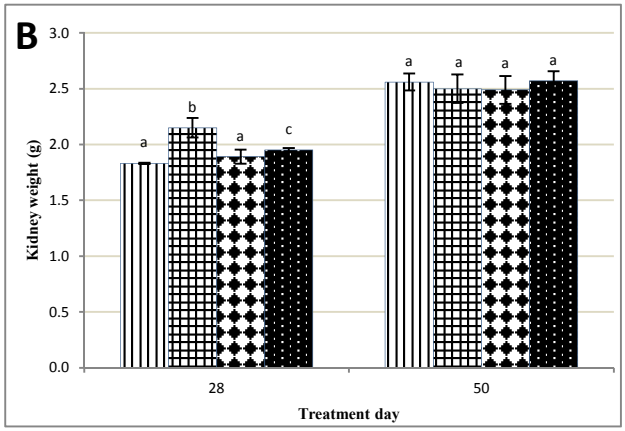


Fig. 5. The effect of supplementation of diet with prebiotics mannan and inulin on weight of hypercholesterolemicmale Wistar rats during 50 days treatment. Control (♦), High cholesterol (■), High cholesterol + mannan (▲), High cholesterol + inulin (●). Means with stars in each day of treatment are significant at 0.05 levels.

The Effect of supplementing different prebiotics on weights of liver, kidney, heart and spleen of rats are shown in Fig.6. It was found that there was no significant increase in liver weight in HC group compared to control after 28 days of feeding. In day 50 of treatment, HC group showed increased liver weight compared to the control ($P<0.05$) but when feeding the HC+M and HC+I diet, they had no difference with the control group (Fig.6A). HC diet induced a significant a weight increase in the kidneys when it was compared to the control group at the 28th day ($P < 0.05$). On day 50 of treatment, kidneys weight of treatment groups were close to control group(Fig.6B).On day 28 and 50 heart weight of rats in all groups was similar (Fig.6C).On day 28 0f experiment spleen weight of HC group was similar to that of control. However, on day 50 HC diet had significantly increased the spleen weight ($P < 0.05$), while this parameter in HC+M and HC+I groups was similar to that of control (Fig.6D). These results showed that dietary inulin and mannan at a dose of 5%were well tolerated by the rats and did not cause over growth and increase in the weight of organ and also reduced the adverse effects of cholesterol in the body weight gain.





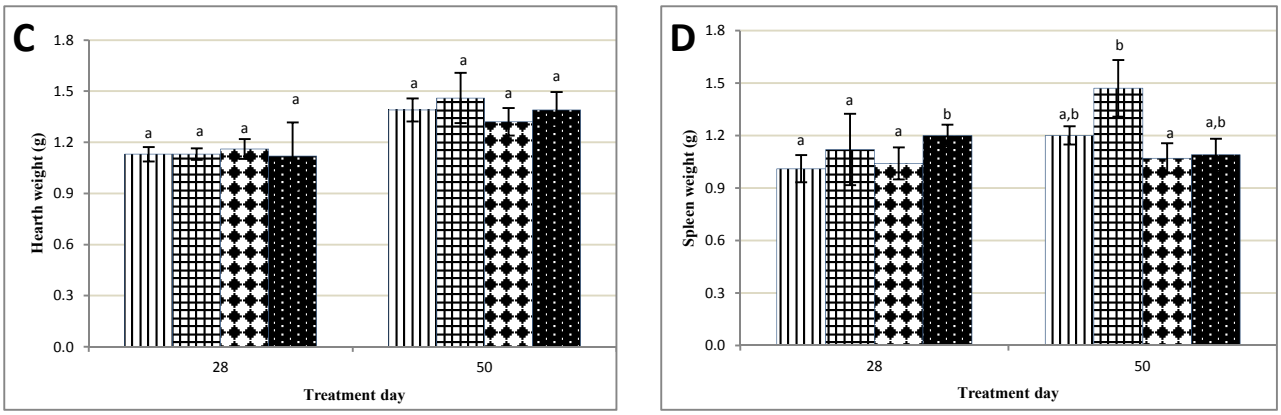


Fig. 6. The effect of supplementation of diet with prebiotics mannan and inulin on A: liver, B: kidney, C: Heart and D: spleen weight of hypercholesterolemicmale Wistar rats. Control ( ), High cholesterol ( ), High cholesterol + mannan ( ), High cholesterol + inulin ( ). Bars represent standard deviation values. Different letters in the same sampling day indicate significant differences at the level of 0.05.

Table1shows the results of glucose tolerance test in rats which was performed before sacrificing. In day 28 and 50, at zero time, blood glucose of HC, HC+M and HC+I groups which was significant compared to the control ($P < 0.05$). Additionally, no significant difference among groups following 120min post-exposure was observed.

Statistical analysis for the effect of feeding different prebiotic on blood glucose at time zero indicates significant increase in HC, HC+M and HC+I groups compare to control group. At time 60 HC diet significantly increased blood glucose but there were no different between groups in time 30 and 90 and 120. A decrease in glucose levels was intended subsequent to the administration of mannan and inulin since many researchers have previously hypothesized the role of fructo-oligosaccharides in the regulation of glucose. For example Delzenne and Kok (1999) had observed that having fructo-oligosaccharides, which are playing a role in the nutritional regulation of lipogenesis, significantly reduces serum insulin and glucose [14]. However, our results were comparable to the study of Causey et al. (2000) on humans who found a close association between an increase in the levels of insulin and glucagon levels following consumption of insoluble fibers [19].

Table 1. The effect of supplementation of diet with prebiotics mannan and inulin on serum glucose (mg/ dL) of hypercholesterolemicmale Wistar rats given glucose solution (5 g/kg).

Day	Treatment groups	Time [min]				
		0	30	60	90	120
28 (n=4)	Control	82.50 ± 2.3^{Aa}	184.50 ± 19.1^{Ba}	178.75 ± 10.6^{Ba}	161.00 ± 8.7^{Ba}	127.00 ± 8.8^{Ca}
	High cholesterol	109.00 ± 4.6^{Ab}	153.00 ± 3.6^{Ba}	168.67 ± 8.8^{Ba}	153.33 ± 13.9^{Ba}	145.00 ± 21.9^{ABa}
	High cholesterol+ mannan	117.75 ± 3.1^{Ab}	160.00 ± 7.8^{Ba}	167.00 ± 7.2^{Ba}	157.00 ± 7.3^{Ba}	134.25 ± 2.5^{Aa}
	High cholesterol+ inulin	99.50 ± 1.3^{Ac}	170.75 ± 10.3^{Ba}	166.75 ± 8.3^{Ba}	165.00 ± 3.2^{Ba}	137.75 ± 9.1^{Ca}
50 (n=3)	Control	93.33 ± 5.8^{Aa}	138.33 ± 10.7^{Ba}	152.67 ± 8.2^{Bab}	144.33 ± 6.0^{Ba}	140.33 ± 7.0^{Ba}
	High cholesterol	110.00 ± 4.5^{Ab}	162.00 ± 9.2^{Ba}	178.33 ± 7.5^{Ba}	180.67 ± 14.5^{Ba}	172.00 ± 11.9^{Ba}
	High cholesterol+ mannan	130.67 ± 3.8^{Ac}	148.33 ± 2.7^{Aa}	143.00 ± 5.6^{Ab}	141.00 ± 6.7^{Aa}	143.33 ± 6.27^{Aa}
	High cholesterol+ inulin	129.00 ± 1.0^{Ac}	158.00 ± 5.9^{ABa}	148.67 ± 11.0^{ABb}	160.67 ± 15.6^{ABa}	165.67 ± 10.5^{Ba}

The different capital letters indicate significant differences in row($P < 0.05$).The different small letters in the same column within the same day indicate significant differences ($P < 0.05$).

In conclusion, our results suggest the imperative role of inulin and mannan to reduce the serum levels of cholesterol, LDL and VLDL. It could be suggested that the prebiotic may be useful and can be used as an alternative or complementary treatment in hyperlipidemia and related disease conditions.

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