Original Article

Effect of the Growth Promotant Mannan-Oligosaccharide on the Lipogram and Organ Function Profile in Hyperlipidemic Albino Rats

Abubakr M. El-Mahmoudy*[1], Fathy A. Abdel-Fattah[2], Afaf D. Abd El-Mageid[3] and Ibtsam M. Gheith[4]

[1]Department of Pharmacology, Benha University Faculty of Veterinary Medicine, Moshtohor, Egypt, 13736
[2]Department of Nutrition and Clinical Nutrition, Benha University Faculty of Veterinary Medicine, Moshtohor, Egypt, 13736
[3]Department of Biochemistry, Benha University Faculty of Veterinary Medicine, 13736 Moshtohor, Egypt, 13736
[4]Department of Biotechnology, Animal Health Research Institute, Dokki, Egypt, 11843

Address for Correspondence

Department of Pharmacology, Benha University Faculty of Veterinary Medicine, Moshtohor, Egypt, 13736.

E-mail: a.elmahmoudy@hotmail.com

ABSTRACT

This study aimed to investigate the effect of mannan-oligosaccharide (MOS) on the lipogram and selected organ functions in hyperlipidemic rats. Animals were subjected to different treatments for 60 days. Balanced basal diet was supplied to control rats; saturated high fat- and/or MOS-contained diets were supplied to tested rats. Sera were collected on escalating times and total lipids, cholesterol, triacylglycerols, HDL-C, LDL-C, VLDL-C, AST, ALT, urea and creatinine were evaluated. On the last day, rats were sacrificed and the kidneys, liver, heart with aorta were picked out for histopathology. The results of MOS-treated group revealed significant decrease in total lipids, cholesterol, LDL-C, VLDL-C, AST, ALT, urea and creatinine with significant increase in HDL-C. Histopathological findings in the tested organs have been ameliorated with MOS, comparing with those in hyperlipidemic rats. The obtained data may give MOS the potential of antihyperlipidemic activity for prophylaxis and treatment of hyperlipidemia and related diseases.

Keywords: Phytomedicine, Hyperlipidemia, Mannan-oligosaccharide, MOS, Prebiotic.

INTRODUCTION

The world Health Organization predicts that by the year 2020, up to 40% of all deaths will be related to cardiovascular disease and associated hyperlipidemia. An increase in serum cholesterol concentration by 1% results in 2-3% increase in the risk of cardiovascular disease[1]. Researchers as[2] concluded that using of antihyperlipidemic chemical drugs as HMG-CoA reductase inhibitors, fibrates, anion–exchange resins (bile acid sequestrants), cholesterol absorption inhibitors, alpha-tocopherol acetate (vitamin E) and omega-3 marine triglycerides, have many side effect including, renal insufficiency, gastrointestinal upsetting effects, hyperuricemia, gout, impaired glucose tolerance, liver dysfunction, myopathy and rhabdomyolysis. In this sense, specialists are saving no effort for getting an alternative remedy for hyperlipidemia without or with minimal side effects.

Recent interest in what is known as nutriceuticals or foods with pharmacological impacts to modulate different abnormal conditions, including blood lipids, such as cholesterol and triglycerides has been developed. Several non-digestible dietary carbohydrates were found able to regulate lipemia and triglyceridemia in both humans and animals with minimal side effects. Prebiotics are one of these carbohydrates and might represent novel therapeutic or preventive agents[3]. The original definition of prebiotics was coined in 1995 by[4] as "non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon as *Bifidibacteria and Lactobacilli*". Some oligosaccharides, 2-10 monosaccharide molecules are joined together to form a larger molecule, fall in this category. Mannose is a monosaccharide that forms the building block of mannan-oligosaccharides (MOS). Mannose-based oligosaccharides occur naturally in cell walls of the yeast *Saccharomyces cerevisiae* and obtained by centrifugation of lysed yeast culture[5]. The small intestine does not contain the digestive enzymes required to break down MOS bonds, therefore they arrive at the large intestine intact after ingestion and passage through the small intestine[6]. In the intestines, MOS provides competitive binding sites for pathogens with mannose-specific type-1 fimbriae such as *Salmonella* and *E. coli* and decreases their attachment with intestinal wall and is ultimately excreted from the gut[7]. In addition, MOS has been demonstrated to be fermented and utilized by and thus increases the cecal populations of beneficial bacteria, namely *Lactobacillus spp.* and *Bifidobacterium spp*[8,9].

The hypolipidemic effects of prebiotics and related compounds as resistant starches and oligosaccharides have received considerable attention as a safe pharmacological approach. Although the mechanism is currently unknown, studies have shown positive results and mechanistic hypotheses have been developed, yet controversial. The focus of this work, therefore, will be to investigate the hyperlipidemia improving profile of the prebiotic MOS as a nutriceutical approach to hyperlipidemia in rats rendered hyperlipidemic by long term feeding on high-fat diet.

MATERIALS AND METHODS

Mannan-oligosaccharide (MOS)

MOS was obtained as the commercial product Bio-Mos®, International Free Trade, Cairo, Egypt; under license from Alltech Inc., Nicholasville, KY, USA. Its active ingredient

is mannose-based oligosaccharide that occurs naturally in the cell walls of the yeast *Saccharomyces cerevisiae* and obtained by centrifugation of lysed yeast culture. The MOS powder was incorporated into the rat diet as 5% (w/w) in the corresponding groups.

Experimental animals

Fifty male rats aging eight weeks of approximate body weight 200 g were used in this study. Rats were kept in suitable cages and allowed to water *ad libitum* and different diets at room temperature. After one week of acclimatization, rats received different treatments as explained below. The European Commission Directive 86/609/EEC for ethics of animal experiments has been followed[10].

Diets

Balanced basal diet was supplied to control rats. High saturated-fat diet was prepared by incorporating coconut oil 2% (w/w) and cholesterol 1% (w/w) into the basal diet. MOS-contained diet was prepared by incorporating MOS 5% (w/w) *viz.* 50 gram/kg diet. Basal diets were formulated according to[11] and illustrated in table 1.

Experimental design

Acclimatized rats were divided into 5 groups, each consists of 10 rats. To fulfill the aim of the present work, groups are subjected to different treatments as follows: Group-1: was negative-control group where rats fed on normal diet and received no drugs; Group-2: was positive hyperlipidemic control group where rats fed on saturated fat-enriched diet and received no drugs; Group-3: was negative MOS-treated group where rats fed on normal diet and received MOS after 30 days from the start of the experiment; Group-4: was positive MOS-treated group where rats fed on saturated fat-enriched diet and received MOS after 30 days from the start of the experiment; and finally Group-5: was positive MOS-prophylactic group where rats fed on saturated fat-enriched diet and MOS from the start of the experiment.

Blood and tissue sampling

Blood for serum was collected on the 30th, 45th and 60th days from the start of the experiment. Samples were collected from the retro-orbital venous plexus located at the medial can thus of the eye by means of heparinized capillary tubes. The collected blood was allowed to clot at room temperature for an hour; and then refrigerated for further an hour for clot retraction. Clear sera were separated by centrifugation at 3000 r.p.m. for 10 minutes and then collected in Eppendorf tubes using automatic pipettes. Serum samples were tested directly for parameters that are protein in nature as enzymes and lipoproteins and then kept in deep freezer (-20 °C) for analysis of the rest of the biochemical parameters that are not affected by freezing and thawing; all tested parameters included TL, TC, TAG, HDL-C, LDL-C, VLDL-C, AST, ALT, urea and creatinine. On the last day of feeding and after blood collection, rats of each group were sacrificed and the two kidneys, liver, heart with aorta were picked out and preserved in formalin solution (10 %) for histopathological examination.

Lipid profile

Serum TL were determined according to the method described by[12]; serum TC was determined enzymatically according to the method described by[13]; serum HDL-C was determined according to the precipitation method described by[14]; serum TAG were determined enzymatically according to the method described by[15]; serum LDL-C value was calculated using the formula described by[14] as LDLC = TC - (HDLC + TAG/5) mg/dL; while serum VLDL-C value was calculated using the formula described by[16] as VLDLC = TAG/5 mg/dL.

Liver function profile

Serum ALT and AST were quantitatively determined according to the methods described by[17,18], respectively.

Kidney function profile

Serum urea was quantitatively determined according to the method described by[19]; while serum creatinine was quantitatively determined according to the method described by[20].

Histopathological examination

The 10% neutral formalin-fixed samples of liver, heart with aorta and kidneys were washed by running water over night. The washed samples were dehydrated using graded ascending concentrations of ethyl alcohol starting with 50% and ending with absolute alcohol. The dehydrated samples were then cleared in xylene for 6 hours. The samples were placed in a crucible containing soft paraffin and kept in an oven at 56°C for 12 hours. The samples were then blocked in hard paraffin and cut into sections of about 5 micrometers in thickness. Paraffin was removed from the sections by two changes of absolute alcohol (five minutes each) which was removed by washing with tap water. Sections were then stained with Harris haematoxylin and eosin for 10 minutes, and then washed with running water for 15 minutes for general histological examination according to[21]. Crossman's trichrome stain was used for the demonstration of the collagenous fibers[22]. The sections were dehydrated again with two changes of absolute alcohol (five minutes each), then cleared with xylene. Sections were mounted with Canada balsam and covered with cover slides to be ready for microscopical examination. Digital photos were picked up to demonstrate the histological changes between groups after different treatments.

Statistical analysis

Data were expressed as mean ± S.E. The obtained data were statistically analyzed using two sample Student's *t*–test to determine the significant differences ($p < 0.05$) between every two groups (first is control and the second is treated) using SPSS computer software[23].

RESULTS AND DISCUSSION

As shown in tables 2 & 3, there were significant increases in serum TL, TC, TAG, LDL-C and VLDL-C concentrations in rats fed on fat-enriched diet, compared to rats received basal diet. While supplementation of MOS-diet to normal rats caused insignificant changes in these parameters all over the period of the experiment; yet, its supplementation significantly decreased the parameters' serum concentrations in rats fed on fat-enriched diet. On the other hand, as shown in table 3, there was a significant decrease in serum HDL-C concentration in rats fed on fat-enriched diet, compared to rats received basal diet. While supplementation of MOS-diet to normal rats caused insignificant changes in serum HDL-C concentration all over the period of the experiment; yet, its supplementation significantly increased serum HDL concentration in rats fed on fat-enriched diet.

As shown in table 4, there were significant increases in serum ALT & AST concentrations in rats fed on fat-enriched diet, compared to rats received basal diet. MOS-diet supplementation significantly decreased the elevated serum ALT & AST concentration in rats fed on fat-enriched diet with insignificant changes in negative control group.

As shown in table 5, there were significant increases in serum urea & creatinine concentrations in rats fed on fat-enriched diet, compared to rats received basal diet. Although supplementation of MOS-diet

to normal rats caused insignificant changes in serum urea & creatinine concentrations; yet, its administration significantly decreased the elevated serum urea & creatinine concentration in rats fed on fat-enriched diet.

There were no remarkable changes in negative control group in the examined organs. However, keeping rats on fat-enriched diet (positive control group) caused some degenerative changes including, fatty infiltration of *Tunica intima* of aorta; hydropic degeneration and periportal fatty change of hepatocytes; and congestion of renal blood vessels with vacuolar, hydropic degeneration and even fatty change of renal tubular epithelium. These changes were ameliorated to a great extent in MOS-treated groups especially in Group-5 whose rats received MOS from the beginning of the experiment (Figures 1-3).

The concept of prebiotics was first introduced in 1995 by[4], and they have gained attention in industry and academia due to their potential health benefits, even became as products in the form of capsules and tablets containing prebiotics. MOS is a non-digestible prebiotic, which modulate intestinal floral activity, exhibit serum cholesterol or triglyceride lowering effect. The mechanism of this effect is currently unclear with four different hypotheses being proposed: the production of propionate, assimilation of cholesterol by bacteria, binding of cholesterol to bacterial cell walls and enzymatic degradation[24].

The present work sheds more light to hyperlipidemia in rats rendered hyperlipidemic by long term feeding of high-fat diet, and investigates the influence use of MOS as a prebiotic in an attempt to improve the generalized elevated lipogram, organ function parameters and pathological lesions induced by hyperlipidemia. In the present study (table 2), there was a significant increase in serum TL concentration in rats fed on fat-enriched diet, compared to rats received basal one. These data were supported by[25]. Such increase occurred as an expected result due to increase of serum TAG, TC, LDL-C and VLDL-C that were noted in this study.

Supplementation of MOS-diet significantly decreased the elevated serum TL concentration in rats fed on fat-enriched diet. Similar results were previously obtained by[26]; but are not in accordance with those reported by[27], who found that the consumption of prebiotics have no significant effects on blood lipids. Although the available data about the mechanistic effects of prebiotics on lipid metabolism are limited, but[28] suggested that prebiotics might decrease lipogenic enzyme activities in the liver and thus decrease lipid concentration in blood. It is possible that such decrease occurs as a result of the decreased levels of serum TAG, TC, LDL-C and VLDL-C that were observed in the treated rats. Moreover[29], suggested that prebiotics may decrease lipogenic enzyme gene expressions. The variety of results of lipid concentration can be attributed to the complexity of the biochemistry of lipid metabolism as mentioned by[30]. Concerning serum TC, there was a significant increase in rats fed on fat-enriched diet, compared to rats kept on basal diet as shown in (table 2), such data go hand in hand with those reported with[25] who reported that rats received cholesterol-enriched diet showed hypercholesterolemia. Rise in serum TC might be attributed to its reduced catabolic rate and/or reduced activity of hepatic cholesterol-7-alpha-hydroxylase, the rate limiting enzyme in bile acid synthesis from cholesterol. Supplementation of MOS-diet to significantly decreased serum TC concentration in rats fed on fat-enriched diet. This result added more support to those previously reported by[31] who mentioned that TC was decreased in prebiotic-given subjects in comparison with those given placebo. On the other hand, this result disagree with[32] who

observed that after 3 months of fructose oligosaccharide as a prebiotic supplementation, no statistically significant differences were observed in TC. Moreover[33] concluded that the prebiotic treatment did not affect hepatic and plasma concentrations of TC[34]. also, reported that consumption of several levels of prebiotics had no significant effect on TC in rats fed a high-fat diet. Negative evidence was also noted by[35] who reported that the prebiotic feeding alone did not influence the serum TC and TAG levels. The most important mechanism by which prebiotic eliminates cholesterol would likely be through reducing lipid absorption in intestine by binding bile acids, which results in increased cholesterol elimination and hepatic synthesis of new bile acid[36]. In addition, this decrease may be related to assimilation of cholesterol by bacteria that a prebiotic stimulates their growth and/or activity in the colon as *Bifidibacteria* and *Lactobacilli*[24].

Assessment of TAG, in the present study, revealed a significant increase in their serum concentration in rats fed on fat-enriched diet, compared to rats kept on basal diet as noted in table (2). The aforementioned results agree with that achieved by[25] who found that a hyperlipidemic diet caused a significant increase of the plasma TAG. Such significant rise in serum TAG level may be related to the decrease of activity of lipase which is an insulin-dependent enzyme involved in TAG clearance from plasma by mediating their lipolysis into glycerol and free fatty acids[37]. Administration of MOS significantly decreased serum TAG concentration in rats fed on saturated fat-enriched diet. Our result is in harmony with that obtained by[38] who achieved that adding 0.08% beta-glucan to diet as a prebiotic to diet, decreased serum TAG against control group. The above result is different from that was reported by[39]. Such decrease in TAG may be attributed to the ability of prebiotics to decrease serum TAG concentration through an extrahepatic event, namely enhancing TAG-rich lipoprotein catabolism[40].

Data obtained in the present study (table 3), revealed a significant decrease in serum HDL-C concentration in rats fed on fat-enriched diet with parallel significant increase in LDL and VLDL, compared to rats received basal di*et al*l over the period of the experiment. These results are fit with those reported by[25] who reported that rats receiving cholesterol-enriched diet showed elevated plasma LDL-C and VLDL-C compared to those kept on a normal diet. These elevations in serum LDL-C and VLDL-C may be related to reduction of their catabolic rate that occurs when the production of LDL exceeds the capacity of LDL receptors present on hepatocytes. Supplementation of MOS together with diet significantly increased serum HDL and decreased LDL-C and VLDL-C concentration in rats fed on saturated fat-enriched diet. A support to our results comes from[41] who found that barel beta-glucan prebiotics increased HDL level in mice; and from[31,42] reported that feeding rats diet supplemented with oligofructose as prebiotics lowered concentrations of VLDL. These results are not in accordance with[32] who observed no statistically significant difference in HDL, after 3 months of fructose oligosaccharides as a prebiotic application. Three mechanisms have been put forward to explain a hypolipidemic effect of prebiotics; the first is the modification of glucose or insulin concentrations where non-digestible carbohydrates reduce peak levels of blood glucose after a meal and consequently the induction of lipogenic enzymes *via* an increased gene transcription[26]. The second mechanism proposes that serum cholesterol is reduced because of precipitation and excretion of bile acids to the intestine, which requires the liver to utilize cholesterol for further bile acid synthesis[43]. The third is that carbohydrates in the colon are fermented to

short chain fatty acids principally, acetate, propionate and butyrate; acetate is mainly metabolized in human muscle, kidney, heart and brain, while propionate is cleared by the liver, and is a possible glucogenic precursor which suppresses cholesterol synthesis. Butyrate, on the other hand, is metabolized by the colonic epithelium where it serves as a regulator of cell growth and differentiation[44].

The activities of ALT and AST were measured as indicators of hepatocellular damage[45]. AST and ALT activities showed significant increase on rats fed on fat-enriched di*et al*l over the experiment when compared to the control rats that received basal diet. These results may attributed to fatty liver disease or/and hepatomegaly as mentioned by[46] and[47], respectively, which occurs as a result of hepatic changes occurred in the hepatic tissue upon fat, and damage of hepatic cell and release of ALT and AST into the blood. This opinion supported by[48] who mentioned that AST and ALT are hepatic enzymes that are released into the blood stream when liver cell are damaged. The fore mentioned results were parallel to the results of histopathological examination which showed hydropic degeneration and periportal fatty change of hepatocytes (figure 1a). MOS-diet supplementation significantly decreased serum ALT & AST concentrations in sera of rats fed on fat-enriched diet. Such decrease of liver enzyme activities is possible after the improvement of microscope lesions of the examined liver as noted in figure 1b.

As for the kidney, as shown in table 5, there was a significant increase in serum urea & creatinine concentrations in rats fed on fat-enriched diet, compared to rats received basal diet, while supplementation of MOS-diet to hyperlipidemic rats significantly decreased serum urea & creatinine concentrations. It was clear that fat-enriched diet caused damage of the renal tissue as clarified by biochemical as well as histopathological means (vacuolar and hydropic degenerations, intertubular edema and hemorrhages and even fatty change of renal tubular epithelium (figure 2a). Renal damage was represented by significant increase in serum urea and creatinine as recorded by[49]. Hyperlipidemia is believed to be followed by lipid accumulation in the renal mesangium with resulting injurious effects such as foam cell formation, cell proliferation, matrix formation and alteration of the charge selectivity in glomerular basement membrane[50]. Supplementation of MOS-diet significantly decreased serum urea & creatinine concentrations in rats fed on fat-enriched diet. This reduction in urea and creatinine concentration may be due to the effect MOS probiotics in improving tissue damage as clarified by both biochemical and histopathological means as noted in figure 2b. Histopathology, in the present study, also showed marked fatty infiltration in the aortic wall that was decreased to a considerable extent in the MOS-treated groups.

CONCLUSION

It could be concluded that the prebiotic MOS may be useful and can be used as an alternative or complementary remedy in hyperlipidemia and related disease conditions.

REFERENCES

1. Manson JE, Tosteson H, Ridker PM, *et al*. The primary prevention of myocardial infarction. *New England Journal of Medicine*. 1992; 326: 1406-16.
2. Kumar AS, Mazumder A and Saravanan US. Antihyperlipidemic activity of Camellia Sinensis leaves in triton wr-1339 induced Albino rats. *Pharmacognosy Magazine*. 2008; 4: 60-4.
3. Rafter J, Bennett M, Caderni G, *et al*. Dietary synbiotics reduce cancer risk factoin polypectomized and colon cancer patients. *American Journal of Clinical Nutrition*. 2007; 85: 488-96.

4. Gibson GR and Roberfroid MB. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *Journal of Nutrition*. 1995; 125: 1401-12.
5. Spring P, Wenk C, Dawson KA and Newman KE. Effect of mannan oligosaccharide on different cecal parameters and on cecal concentration of enteric bacteria in challenged broiler chickens. *Poultry Science*. 2000; 79: 205-11.
6. Strickling JA, Harmon DL, Dawson KA and Gross KL. Evaluation of oligosaccharide addition to dog diets influences on nutrient digestion and microbial populations. *Animal Feed Science and Technology*. 2000; 86: 205-19.
7. Ferket PR, Parks CW and Grimes JL. Benefits of dietary antibiotic and Mannan-oligosaccharides supplementation for poultry. *Proceedings of Multi-State Poultry Feeding and Nutrition Conference*. Indiana, USA: *Indianapolis*, 2002, p. 14-6.
8. Yang Y, Iji PA, Kocher A, Mikkelsen LL and Choct M. Effects of mannan oligosaccharide on growth performance, the development of gut microflora, and gut function of broiler chickens raised on new litter. *Journal of Applied Poultry Research*. 2007; 16: 280-8.
9. Baurhoo B, Phillip L and Ruiz-Feria CA. Effects of Purified Lignin and Mannan-oligosaccharides on Intestinal Integrity and Microbial Populations in the Ceca and Litter of Broiler Chickens. *Poult Sci*. 2007; 86: 1070-8.
10. European commission. Legislation for the protection of animals used for scientific purposes. 2010.
11. NRC. *Nutrient Requirements of Laboratory Animals*. 4th ed.: Subcommittee on Laboratory Animal Nutrition, Committee on Animal Nutrition, Board on Agriculture, National Research Council, 1995, p.192.
12. Chabrol E and Castellano A. SPV method for estimation of total serum lipid. *Journal of Laboratory Clinical Medicine*. 1961; 57: 300.
13. Meiattini F. The 4-hydroxybenzoate/4-aminophenazone chromogenic system. *Clinical Chemistry*. 1978; 24: 2161-5.
14. Friedewald WT, Levy RI and Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the ultracentrifuge. *Clinical Chemistry*. 1972; 18: 449–502.
15. Young D and Pestaner L. Effects of disease on clinical laboratory tests. *Clinical Chemistry*. 1975; 21: 5.
16. Bauer J, D. *Clinical laboratory methods*. Missouri: the C.V. Company Waistline Industrial, 1982.
17. Murray R. Alanine aminotransferase. In: Kaplan A and Peace AL, (eds.). *Clinical Chemistry*. St Louis, Toronto, Princeton: The C. V. Mosby Co., 1984, p. 1088-90.
18. Murray R. Aspartate aminotransferase. In: Kaplan A and Peace AL, (eds.). *Clinical Chemistry*. St Louis, Toronto, Princeton: The C.V. Mosby Co., 1984, p. 1112-16.
19. Kaplan A. Urea. In: Kaplan A and Peace AL, (eds.). *Clinical Chemistry*. St Louis, Toronto, Princeton: The C.V. Mosby Co., 1984, p. 1257-60, 437, 418.
20. Murray R. Creatinine. In: Kaplan A and Peace AL, (eds.). *Clinical Chemistry*. St Louis, Toronto, Princeton: The C.V. Mosby Co., 1984, p. 1261-66, 418.
21. Drury R and Wallington E. *Carlton's Histological Techniques*. 5th ed. Oxford,New York and Toronto: Oxford Unvi. Press, 1980.
22. Crossman GA. A modification of Mallory's connective tissue stain with discussion of principles involved. *Anatomical Record*. 1937; 69: 33-8.
23. SPSS Inc. SPSS v20 for Windows. Chicago, IL, USA2011.
24. Pereira DIA and Gibson GR. Effects of consumption of probiotics and prebiotics on serum lipids levels in humans. *Critical Reviews in Biochemistry and Molecular Biology*. 2002; 37: 259-81.
25. El-Mahmoudy A, Shousha S, Abdel-Maksoud H and AbouZaid O. Effect of long-term administration of sildenafil on lipidprofile and organ functions in hyperlipidemic rats. *Acta Biomed*. 2013; 84: 12-22.
26. Roberfroid M. Functional food concept and its application to prebiotics. *Digestive and Liver Disease*. 2002; 34: 105-10.

27. Van Dokkum W, Wezendonk B, Srikumar TS and van den Heuvel EG. Effect of nondigestible oligosaccharides on largebowel functions, blood lipid concentrations and glucose absorption in young healthy male subjects. *European Journal of Clinical Nutrition*. 1999; 53: 1-7.
28. Delzenne NM and Kok N. Effect of non-digestible fermentable carbohydrates on hepatic fatty acid metabolism. *Biochemical Society Transactions*. 1998; 26: 228-30.
29. Delzenne NM and Kok N. Effects of fructans-type prebiotics on lipid metabolism. *American Journal of Clinical Nutrition*. 2001; 73: 456-8.
30. Van Loo JAE. Prebiotics promote good health. The basis, the potential and the emerging evidence. *Journal of Clinical Gastroenterology*. 2004; 38: 70-5.
31. Davidson MH, Maki KC, Synecki C, Torri SA and Drennan KB. Effects of dietary inulin on serum lipids in men and women with hypercholesterolemia. *Nutrition Research*. 1998; 18: 503-17.
32. Boutron-Ruault MC, Marteau P, Lavergne-Slove A, *et al*. Effects of a 3-mo consumption of short-chain fructo-oligosaccharides on parameters of colorectal carcinogenesis in patients with or without small or large colorectal adenomas. *Nutr Cancer*. 2005; 53: 160-8.
33. van Meer H, Boehm G, Stellaard F, *et al*. Prebiotic oligosaccharides and the enterohepatic circulation of bile salts in rats. *American Journal of Physiology Gastrointestinal and Liver Physiology*. 2008; 294: G540-7.
34. Ahmdifar E, Akrami R, Ghelichi A and Zarejabad AM. Effects of different dietary prebiotic inulin levels on blood serum enzymes, hematologic, and biochemical parameters of great sturgeon (Huso huso) juveniles. *Comparative Clinical Pathology*. 2011; 20: 447-51.
35. Mallikarjuna PVR, Kondal Reddy K and Sashidhar RB. Assessment of health benefits of membrane extracted innate plant fructoprebiotics and lacto-probiotics in rats. *Journal of Microbiology and Biotechnology Research*. 2012; 5: 698-702.
36. Zhang WF, Li DF, Lu WQ and Yi GF. Effects of isomalto-oligosaccharides on broiler performance and intestinal microflora. *Poultry Science*. 2003; 82: 657-63.
37. Yost TJ, Froyd KK, Jensen DR and Eckel RH. Change in skeletal muscle lipoprotein lipase activity in response to insulin/glucose in non-insulin-dependent diabetes mellitus. *Metabolism*. 1995; 44: 786-90.
38. Imanpour-Jodey S, Moghaddaszadeh-Ahrabi S and Rezapour A. The effects of saccharomyces cervisiae beta-glucan on blood lipids in broiler chickens. *Annal Biol Res*. 2013; 4: 134-7.
39. Aubin MC, Lajoie C, Clément R, Gosselin H, Calderone A and Perrault LP. Female rats fed a high-fat diet were associated with vascular dysfunction and cardiac fibrosis in the absence of overt obesity and hyperlipidemia: therapeutic potential of resveratrol. *Journal of Pharmacology and Experimental Therapeutics*. 2008; 325: 961-8.
40. Kok N, Taper H and Delzenne NM. Oligofructose modulates lipid metabolism alterations induced by a fat-rich diet in rats. *Journal of Applied Toxicology*. 1998; 18: 47-53.
41. Hong Zhen N, Ying Li L and T. Y. *Modern Preventive Medicine*. 2009.
42. Kok N, Roberfroid M and Delzenne N. Involvement of lipogenesis in the lower VLDL secretion induced by oligofructose in rats. *British Journal of Nutrition* 1996; 76: 881-90.
43. Pedersen A, Sandstorm B and Vanamelsoort JM. The effect of ingestion of inulin on blood lipid and gastrointestinal symptoms in healthy femals. *British Journal of Nutrition*. 1997; 78: 215-22.
44. Cummings JH. Short chain fatty acids. In: Gibson GR and Macfarlane GT, (eds.). *Human colonic bacteria: Role in Nutrition, Physiology and Pathology*. Boca Raton, FL: CRC Press, 1995.
45. Harr KE. Clinical chemistry of companion avian species: A review. *Veterinary Clinical Pathology*. 2002; 31: 143-51.
46. Altunkaynak BZ. Effects of high fat diet induced obesity on female rat livers (a

histochemical study). *European Journal of General Medicine*. 2005; 2: 100-9.
47. Oldenburg B and Pijl H. Abdominal obesity: metabolic complications and consequences for the liver. *Nederlands tijdschrift voor geneeskunde*. 2001; 145: 1290–4.
48. Fujii T. Toxicological correlation between changes in blood biochemical parameters and liver histopathological findings. *Journal of Toxicological Science*. 1997; 22: 161-83.
49. Kaneko JJ. *Clinical biochemistry of domestic animals*. 5th ed. San Diego (CA): Academic Press, 1997.
50. Shohat J and Boner G. Role of lipids in the progression of renal disease in chronic renal failure: evidence from animal studies and pathogenesis. *Israil Journal of Medical Science*. 1993; 29: 228-39.

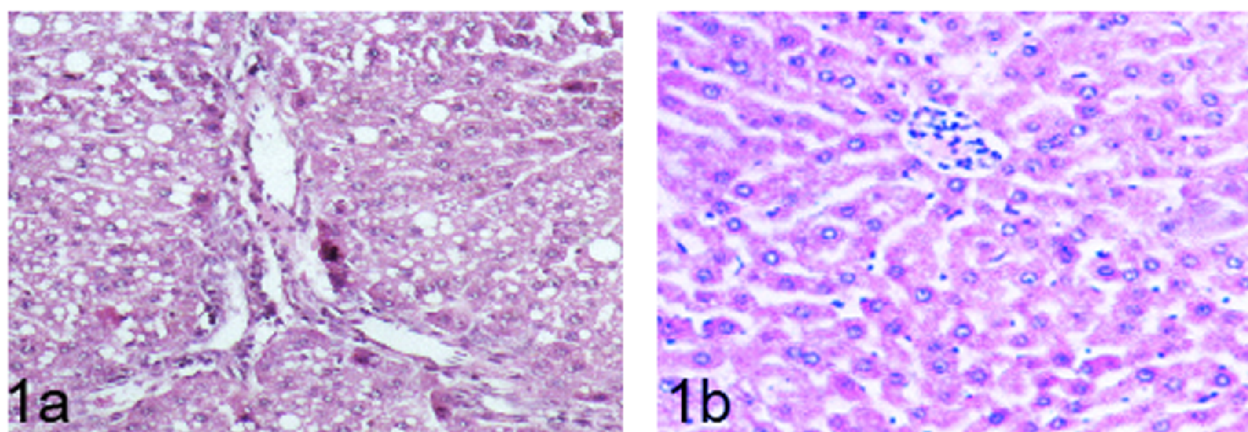

Figure 1. Effect of MOS 5% daily supplementation on the histological structure of the liver of albino rats fed on fat-high diet; a, high-fat control; b, high-fat MOS-treated (n=10)

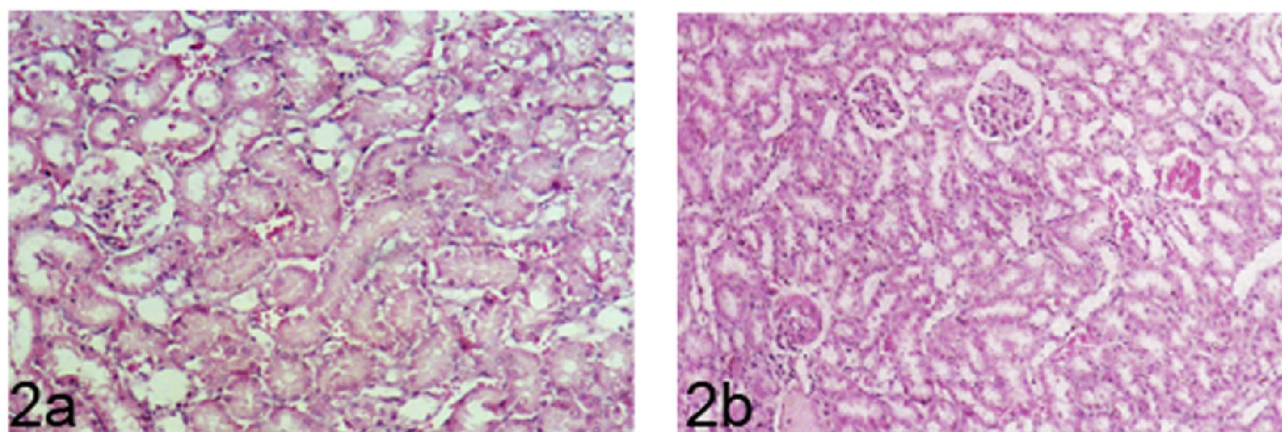

Figure 2. Effect of MOS 5% daily supplementation on the histological structure of the kidney of albino rats fed on fat-high diet; a, high-fat control; b, high-fat MOS-treated (n=10)

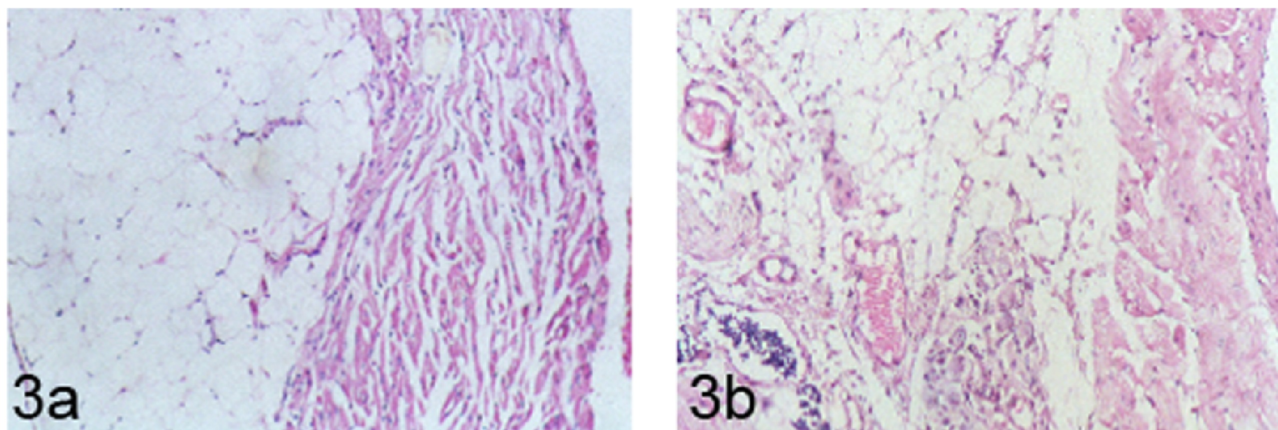

Figure 3. Effect of MOS 5% daily supplementation on the histological structure of the aorta of albino rats fed on fat-high diet; a, high-fat control; b, high-fat MOS-treated (n=10)

Table 1. Composition of the basal and saturated fat-enriched diets for rats (%)

Feed ingredients	Basal diet	Saturated fat & cholesterol -enriched diet	Basal diet with added bio-MOS	Saturated fat & cholesterol -enriched diet with bio-MOS
Bio-MOS	00.00	00.00	05.00	05.00
Oil[1]	14.00	12.00	18.00	16.00
Yellow corn	54.00	55.70	43.00	44.70
Concentrate mixture[2]	05.00	05.00	05.00	05.00
Soya bean meal (44%)	15.50	13.00	17.50	15.00
Wheat bran	05.00	05.00	05.00	05.00
Cholesterol	00.00	01.00	00.00	01.00
Coconut oil	00.00	02.00	00.00	02.00
Molasses	03.00	03.00	03.00	03.00
Common salt	00.50	00.50	00.50	00.50
Lysine	00.20	00.20	00.20	00.20
DL-methionine	00.70	00.70	00.70	00.70
Min.-vit. premix[3]	01.50	01.50	01.50	01.50
Ground limestone	00.60	00.40	00.60	00.40
Total	100.00	100.00	100.00	100.00
Calculated analysis				
Crude protein %	15.12	15.13	15.10	15.02
ME (kcal/kg diet)[4]	3987.5	3982.1	3965.5	3960.1
Calcium	0.55	0.56	0.55	0.57
Phosphorus	0.45	0.48	0.44	0.47
Crude fiber	2.63	2.52	2.52	2.41
Lysine	0.95	0.94	0.97	0.97
Methionine	1.00	1.03	0.99	1.02

[1]**Based on 1g** = 8.5 kcal (Small Animal Nutrition, 2001. Sandi Agar. Elsevier Limited. British Library Cataloguing in Publication Data. Butterworth Heinemann, Oxford).
[2]**Concentrate mixture**; composed of corn gluten 60%, meat and bone meal 50%, sunflower meal 44%, fish meal 45%, dry yeast, limestone, broiler premix, L-lysine HCl, Dicalcium phosphate, bone meal, Choline chloride, DL-methionine, and common salt (Royal Food, Sharqia, Egypt).
[3]**Each 3 kg contains**; Vitamins (A=12000000 IU, D_3= 2000000 IU, E= 10000 mg, K_3= 2000 mg, B_1= 1000 mg, B_2= 5000 mg, B_6= 1500 mg, B_{12}= 10 mg, Biotin= 50 mg, pantothenic acid= 10000 mg, nicotininc acid= 30000 mg, and folic acid= 1000 mg); Minerals (manganese= 60000 mg, zinc= 50000 mg, iron= 30000 mg, copper= 10000 mg, selenium= 100 mg, and cobalt= 100 mg); and carrier ($CaCo_3$) added to 3 kg. Produced by AGRI-Vet. Tenth of Ramadan City, A2, Egypt**.**
[4]**Metabolizable Energy.**

Table 2. Effect of MOS 5% daily supplementation on serum total lipids, total cholesterol and triacylglycerls ($\bar{X}$ ± S.E; n=10)

Parameter	Sample time	Group 1, -ve control			Group 2, +ve control			Group 3, –ve treated			Group 4, +ve treated			Group 5, +ve prophylactic		
Total lipids (mg/dL)	Day 30	321.2	±	17.3	677.7*	±	22.2	325.6	±	20.5	669.5	±	23.6	499.2¤	±	18.5
	Day 45	345.7	±	25.5	692.6*	±	24.3	335.8	±	23.9	590.6¤	±	24.8	511.6¤	±	20.9
	Day 60	373.1	±	26.7	711.2*	±	25.1	347.6	±	25.7	550.8¤	±	26.4	522.8¤	±	22.2
Total cholesterol (mg/dL)	Day 30	68.2	±	6.6	142.3*	±	9.6	73.6	±	5.5	141.5	±	7.9	125.6¤	±	8.3
	Day 45	72.2	±	5.4	163.5*	±	7.9	70.1	±	5.1	128.6¤	±	7.2	112.3¤	±	4.4
	Day 60	78.8	±	8.6	170.1*	±	13.1	66.9	±	9.7	115.3¤	±	7.5	101.2¤	±	7.1
Triacylglycerols (mg/dL)	Day 30	88.7	±	5.7	186.6*	±	8.6	86.11	±	3.6	180.5	±	7.4	121.2¤	±	9.1
	Day 45	92.5	±	4.2	189.62*	±	8.6	83.3	±	5.1	132.01¤	±	9.8	110.3¤	±	8.6
	Day 60	85.1	±	6.1	198.5*	±	7.4	80.8	±	4.1	122.21¤	±	4.4	103.3¤	±	10.1

*significantly different from group-1; ¤significantly different from group-2 ($p<0.05$)

Table 3. Effect of MOS 5% daily supplementation on serum HDL-C, serum LDL-C and VLDL-C ($\bar{X}$ ± S.E; n=10)

Parameter	Sample time	Group 1, –ve control			Group 2, +ve control			Group 3, –ve treated			Group 4, +ve treated			Group 5, +ve prophylactic		
Serum HDL-C (mg/dL)	Day 30	41.8	±	3.3	25.3*	±	2.5	43.3	±	2.3	24.9	±	3.9	33.5¤	±	2.2
	Day 45	42.3	±	4.5	21.5*	±	2.8	45.1	±	2.1	28.5¤	±	4.2	35.5¤	±	2.6
	Day 60	41.1	±	2.1	17.9*	±	2.9	47.2	±	3.1	32.5¤	±	4.6	39¤	±	2.9
Serum LDL-C (mg/dL)	Day 30	17.8	±	1.6	63.6*	±	3.6	14.5	±	1.9	64.79	±	3.2	58.9¤	±	2.1
	Day 45	25.6	±	1.4	75.3*	±	4.5	23.5	±	2.2	55.2¤	±	3.6	51.2¤	±	3.3
	Day 60	30.1	±	1.9	82.3*	±	5.1	27.2	±	3.3	50.2¤	±	4.9	45.5¤	±	2.1
Serum VLDL-C (mg/dL)	Day 30	14.5	±	1.9	25.9*	±	2.3	12.6	±	2.3	26.8	±	2.5	24.2¤	±	2.5
	Day 45	15.9	±	1.8	27.5*	±	2.3	13.5	±	2.2	24.3¤	±	2.3	21.3¤	±	1.9
	Day 60	16.1	±	2.2	29.6*	±	2.2	14.5	±	2.8	23.1¤	±	3.6	18.9¤	±	2.7

*significantly different from group-1; ¤significantly different from group-2 ($p<0.05$)

Table 4. Effect of MOS 5% supplementation on serum ALT & AST activities ($\bar{X}$ ± S.E; n=10)

Parameter	Sample time	Group 1, –ve control			Group 2, +ve control			Group 3, –ve treated			Group 4, +ve treated			Group 5, +ve prophylactic		
AST (U/L)	Day 30	23.1	±	2.3	38.7*	±	2.4	22.5	±	2.9	37.7	±	2.6	33.3¤	±	1.6
	Day 45	21.7	±	2.5	39.3*	±	3.3	21.1	±	2.2	33.6¤	±	2.1	32.3¤	±	2.1
	Day 60	20.9	±	2.6	39.8*	±	3.1	21.2	±	3.1	32.1¤	±	1.4	30¤	±	2.2
ALT (U/L)	Day 30	14.4	±	2.1	25.8*	±	1.4	16.1	±	1.3	26.3	±	1.3	21.5¤	±	1.25
	Day 45	16.3	±	1.6	27.6*	±	2.3	15.6	±	1.2	23.1¤	±	2.5	20.7¤	±	1.84
	Day 60	15.7	±	1.8	28.2*	±	1.6	15.4	±	1.5	21.5¤	±	1.4	20.1¤	±	1.84

*significantly different from group-1; ¤significantly different from group-2 (p<0.05)

Table 5. Effect of MOS 5% daily supplementation on serum creatinine and urea concentrations ($\bar{X}$ ± S.E; n=10)

Parameter	Sample time	Group 1, –ve control			Group 2, +ve control			Group 3, –ve treated			Group 4, +ve treated			Group 5, +ve prophylactic		
Creatinine (mg/dL)	Day 30	1.12	±	0.07	1.85*	±	0.08	1.25	±	0.06	1.75	±	0.06	1.52¤	±	0.08
	Day 45	1.25	±	0.08	1.93*	±	0.09	1.31	±	0.07	1.65¤	±	0.07	1.34¤	±	0.07
	Day 60	1.18	±	0.09	2.11*	±	0.1	1.33	±	0.1	1.53¤	±	0.09	1.33¤	±	0.09
Urea (mg/dL)	Day 30	40.6	±	2.9	61.1*	±	3.1	39.5	±	1.8	58.1	±	1.9	50.2¤	±	2.4
	Day 45	43.5	±	1.8	60.8*	±	1.8	39.1	±	2.5	53.1¤	±	2.1	47.6¤	±	2.6
	Day 60	44.1	±	2.5	62.3*	±	2.5	37.5	±	1.7	49.9¤	±	2.2	45.6¤	±	2.3

*significantly different from group-1; ¤significantly different from group-2 (p<0.05)