Lipids Profiles And Antioxidants Status Of Male Rabbits Fed With Chitosan And Lovastatin

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Abstract
The present study aimed to compare the effect of feeding male rabbits with chitosan or lovastatin on the lipids profiles and antioxidant concentration. Rabbits were fed with chitosan (0.25 g/kg and 0.50 g/kg body weight) or lovastatin (3.5 mg/kg body weight) through a standard laboratory diet for 60 days. Results revealed that diet supplementation with 0.25 g/kg and 0.50 g/kg chitosan and 3.5 mg/kg lovastatin significantly reduced serum cholesterol, triglycerides, low-density lipoproteins, very low-density lipoproteins, and glutathione, compared to the control. Treatment with chitosan and lovastatin in normal rabbits increased the level of high-density lipoprotein compared to the control. However, the activity of aspartate aminotransferase (AST) and alanine transaminase (ALT), and the concentration of malaldehydedihydrate (MDA) were significantly decreased in rabbits fed with chitosan compared to those fed with lovastatin or the control. Thus, the current study demonstrates that chitosan possesses a hypolipidemic effect and improved the antioxidant concentration in the serum of male rabbits.
Keywords: Chitosan; Lovastatin; Dietary supplementation; Rabbits; Hypolipidemic effect.

Introduction

Cardiovascular and chronic diseases are often associated with impaired lipid metabolism (Kaabia et al., 2018; Liu et al., 2021). Word health organization (WHO) has reported that over 38% of people were overweight, and fatness is one of the major risk factors for fatty liver disease (WHO, 2020). Fernandez et al., (2001) showed that hypercholesterolemia is a risk factor for heart disease. In this vein, animal models are useful for deciphering the physiological mechanisms underlying such pathologies.

Chitosan oligosaccharides are oligomers of D-glucosamine produced from enzyme hydrolysis of chitin or by acid hydrolysis (Lodhi et al., 2014), which have good water solubility and are easily absorbed by the body (Xia et al., 2011). In addition to chitosan's enhanced role in immunity (Nelson et al. 1994), it was also suggested to be used to reduce the concentration of fats and cholesterol (Chien and Chou 2006). In this vein, some researchers have incorporated chitosan in weight loss programs or to treat overweight cases (Mulder et al. 2015). Gallaher et al., (2000) showed that chitosan is chemically similar to cellulose, is not digestible by digestive enzymes, and acts as a dietary fibre in the gut tract. Moreover, many studies showed that chitosan feeding inhibits dietary fat digestion and reduces serum cholesterol levels (Yao and Chiang, 2005; Liu et al., 2018; Chiu et al., 2019). Whereas lovastatin is a secondary metabolite commonly produced by filamentous fungi and has various applications in the health and agricultural industries. Preliminary studies on this molecule showed its ability to bind

to the active site of the HMG-CoA enzyme, which determines the first limiting step in the cholesterol biosynthesis pathway (Mulder et al. 2015). Thus, it is likely to regulate cholesterol biosynthesis. Therefore, this study aimed to compare the effectiveness between chitosan and lovastatin as antioxidants tending to lower cholesterol levels in male rabbits.

Material and Methods

Animals, management and diets

For this study, male rabbits (n = 24) were obtained from the Veterinary Hospital of the Ministry of Agriculture, Samarra. They were 4-5 months-aged animals, 1710 ± 0.645 g average weight, placed in metal cages (19×21×25 cm) and kept under field conditions at 22°C, for a light period of 8 hours left for adaptation before starting the experiment. The animals were divided into 4 groups (6 animals per group), namely, control diet (treatment 1); 0.25 g/kg chitosan added to the control diet (treatment 2); 0.50 g/kg chitosan added to the control diet (treatment 3); and 3.50 mg/kg lovastatin added to the control diet (treatment 4). The control diet consisted of a balanced food based on crushed 30%, yellow corn 30%, soybeans 25%, wheat bran 12.3%, vitamins 0.2%, limestone 2%, food salt 0.5, protein content 18.78%, and energy 2514.4 kcal/kg. Animals were watered and fed daily throughout the duration of the experiment.

Blood sample extraction

Blood samples were collected from the rabbits' hearts using a heart stab with an 8 ml wine syringe after 30 and 60 days of treatment. The samples were centrifuged at 4000 rpm for 15 minutes, blood serum was separated from the remaining blood components, and stored at minus 20°C until performing the analysis.

Lipids, liver enzymes and antioxidant activities quantification

Serum cholesterol, triglycerides, and high-density lipoproteins (HDL) were determined through the enzymatic method using the commercial kit REF 11505 (Biomeriux, Biolabo, France). The low-density lipoproteins (LDL) were measured according to Friedewald et al. (1972), with the following equation: LDL concentration (mg/100 ml blood) = total cholesterol - (HDL + triglycerides). The concentration of very low-density lipoproteins (VLDL) in serum was determined according to the following equation: VLDL concentration (mg/100 ml blood) = triglycerides/5.

The concentration of liver enzymes, including aspartate aminotransferase (AST), alkaline phosphatase (ALP) and alanine transaminase (ALT) was calculated calorimetrically according to Reitman (1957). The concentration of urea and creatinine was determined using a spectrophotometer (type pd 303, Apel, India) and a ready-to-use kit (BioSystems, Linear Chemicals, Spain) following Young's (1997) instructions.

The concentration of glutathione was quantified following the method reported by Al-Zamely, Al-Nimer and Al-Muslih (2001) with modifications. Briefly, the method allows to estimate the reduced glutathione in serum; it uses Elman's reagent that contains 5,5-Dithio bis-2-Nitrobenzoic acid (DTNB), which strongly reacts with the sulfhydryl group of the glutathione, which is reduced yielding a yellow complex that absorbs at 412 nm. Whereas the concentration of malaldehydedihydrate (MDA) was determined according to Muslih, Al-Nimer and Al-Zamely (2002).

Statistical analyses

The assays were performed using a complete randomized design (CRD) in one way. Data were analysed using the software Statistical Analysis System version 9 (SAS, 2004). ANOVA analyses were performed to detect significant variances and were followed by Duncan's multiple range test (Duncan, 1955) at a 95% or 99% confidence level.

Results

Lipids profiles

We observed significant differences ($p \leq 0.01$) in the level of lipids profiles in the blood of rabbits that were fed with both doses of chitosan and lovastatin after 30 and 60 days of the treatment (Table 1). The highest and most significant decrease in the cholesterol level ($p \leq 0.01$) was recorded in the group of rabbits fed with 3.5 mg/kg lovastatin (treatment 4) and 0.50 g/kg chitosan (treatment 3), which recorded an average reduction during the 30 and 60 days period, respectively, compared to the control (treatment 1). Whereas the group fed with 0.25 g/kg chitosan (treatment 2) experienced a slighter reduction (by 119.77 mg/dL on average between both periods) but was still significant compared to the control.

The triglyceride level was lowered, on average in the groups of rabbits fed with both doses of chitosan, during the 30 and 60 days period, respectively, and no significant differences were detected between them. Whereas the treatment with lovastatin lowered the level of triglycerides, compared to the control group.

The concentration of HDL was significantly increased ($p < 0.01$) in the group of rabbits that received 0.50 g/kg chitosan and in the group of rabbits that received 3.5 mg/kg lovastatin only during 60 days of the experiment, compared to the control. No significant differences were detected in HDL levels for the rabbits fed with any of the other treatments (Table 1).

The concentration of LDL was significantly decreased by all the treatments with chitosan and lovastatin during the periods of 30 and 60 days, compared to the control (Table 1). The best effect was detected in rabbits fed with 0.50 g/kg chitosan and 3.5 mg/kg lovastatin, which lowered the concentration of LDL after 30 days and after 60 days, respectively, compared to the control. A minor effect, but still significant, was observed in rabbits fed with 0.25 g/kg chitosan, which presented a reduction of LDL levels after 30 days and after 60 days, compared to the control.

Whereas the most significant decrease in the blood serum level of VLDL ($p < 0.01$) was detected in rabbits fed with 0.50 g/kg chitosan and 3.5 mg/kg lovastatin, followed by those who received 0.25 g/kg chitosan compared to the control after 30 days of treatment (Table 1). Such a significant effect of both chitosan and lovastatin persisted even after 60 days, except for those rabbits fed with 0.5 g/kg chitosan who experienced a significant decrease in VLDL levels by that time ($p < 0.01$), compared to the control.

Liver enzymes activity

The activity of the liver enzymes AST and ALT were significantly affected by some of the doses of chitosan and lovastatin after 30 and 60 days of treatment (Figure 1). Regarding AST, both doses of chitosan reduced its activity, but only the highest dose (treatment 3) had a significant effect, reducing AST activity after 30 and 60 days of treatment, respectively, compared to the

control (Figure 1a). Whereas an increase in the activity was induced by lovastatin, yet, was not significant for any of the times analysed. A similar trend was observed for ALT activity, except that neither of the treatments induced a significant effect compared to the control (Figure 1b).

Glutathione and Malondialdehyde concentrations

The serum levels of glutathione and malondialdehyde (MDA) were only significantly affected in those rabbits that received the lowest doses of chitosan in the diet after 30 and 60 days of treatment (Figure 2). In this vein, the level of glutathione was similarly increased by treatment 2 (0.25 g/kg chitosan) after 30 and 60 days of treatment compared to the control, whereas neither of the other treatments induced a significant change (Figure 2a). Regarding the level of MDA, it was significantly decreased after 30 and 60 days of treatment, respectively, in those rabbits that received 0.25 g/kg chitosan, compared to the control (Figure 2b).

Discussion

Chitosan and lovastatin improve the serum levels of lipids in rabbits

In the present study, we demonstrated that chitosan could positively modulate the serum level of lipids. In general, the reduction in cholesterol, LDL and VLDL levels was significantly greater in those rabbits fed with the higher dose of chitosan (0.50 g/kg) and similar to that achieved with lovastatin (3.5 mg/kg) when administered in the diet of male rabbits. Chitosan can also attract negatively charged fatty acids (fatty acids or bile acids) forming a complex that is excreted out of the intestine and from the body (Muzzarelli et al., 2006; Zhou et al., 2006). Studies have confirmed that chitosan affects adipocytokines such as the C-reactive protein, Leptin, and Resistin, which have a major role in regulating energy metabolism and preventing fat deposition in the liver (Unger 2003a,b). Whereas the reduction achieved using lovastatin may be explained due to the ability of such a drug to inhibit the action of the enzyme HMG-CoA reductase, which has a major role in producing cholesterol in the body leading to a reduction in cholesterol, LDL, VLDL and an increasing in the levels of good cholesterol HDL (Mulder et al. 2015).

The incorporation of chitosan into the rabbit's diet, indistinct its concentration, also led to the reduction of the levels of triglycerides. It is known chitosan reduces the formation of triglycerides in the liver, reducing the deposition of fat in the body without affecting the digestibility of fats in the intestine. Similar results were attained using different animals. For instance, pigs fed with a diet supplemented with 0.025% chitosan showed a reduction in blood serum levels of total cholesterol and triglycerides (Tang et al., 2005). Mice fed with a high-fat content diet supplemented with 5% chitosan experienced a significant reduction in the level of total cholesterol and triglycerides in serum compared to the control group (Neyrinck et al., 2009). Rats fed with a basic and high-fat diet supplemented with either 5% chitosan or a mixture of chitosan and wheat bran, led to the reduction of cholesterol, triglycerides, LDL, and VLDL levels, and the increment in the level of lipoproteins (Lamiaa 2011). Hamsters fed with a high-fat diet (20% soybean oil) supplemented with two doses of chitosan (5 mg/kg or 10 mg/kg of body weight) showed a significant reduction in the level of total cholesterol, triglycerides and LDL and VLDL, while no significant

effect was observed in the level of HDL after 12 weeks (Wu et al. 2012). Whereas Jersey heifers fed with chitosan (2 g/kg of dry matter feed) experienced a significant reduction in the level of total cholesterol and LDL, and an increase in HDL, compared to the control group (Gandra et al. 2016). Interestingly, chitosan also exerts a positive effect on the level of lipids of rats suffering from diabetes (Abdel-Rahim et al., 2016). Authors induced diabetes in rats using alloxan, which were further fed with chitosan 5% in feed and 5 g dissolved in water and observed such animals experienced a significant reduction in the level of total cholesterol, triglycerides and LDL, and an increase in HDL, compared to animals with diabetes.

Chitosan has also been tested in humans achieving promising results. People who suffered from high blood lipids received a treatment based on two types of chitosan (300 mg), one dissolved in water and the other insoluble for 8 weeks, and experienced a significant reduction in the level of total cholesterol and LDL compared to the placebo group (Liao et al. 2007).

In general, neither of the chitosan or lovastatin treatments affected the activity of the liver enzymes AST and ALT, except that the highest dose of chitosan (g/kg) induced a slight but significant decrease in the activity of AST. These results indicate that chitosan and lovastatin did not have any negative or toxic effects on the treated animals. Similar to us, Mohamed (2011) found that feeding rats with a high-fat diet supplemented with chitosan 5% led to a decrease in the level of the enzymes AST and ALT, compared to the positive control group. Abdel-Rahim et al. (2016) reached a similar result; they observed a significant decrease in the transporting enzymes for the group of amines, AST and ALT in rats with induced diabetes when fed with chitosan (5% in feed and 5g dissolved in water) compared to the control groups.

Chitosan and lovastatin increase the serum antioxidant activity in rabbits

The results achieved in the current study agree with many studies in which the biological effect of chitosan was demonstrated regarding oxidation and its antioxidation. For instance, Mohamed (2011) found that the treatment of induced oxidative stress with chitosan led to a significant increase in the level of glutathione (GSH) and catalase (CAT) compared to the group affected by oxidative stress. Moreover, he reported that rats fed with a high-fat diet supplemented with 5% chitosan led to a decrease in MDA and an increase in GSH (Mohamed 2011). Likewise, rats fed with a basic and high-fat diet supplemented with either 5% chitosan or a mixture of chitosan and wheat bran, led to a decrease in MDA and an increase in GSH (Lamiaa, 2011). Whereas in rats with induced diabetes fed with chitosan (5% in feed and 5g dissolved in water), Abdel-Rahim et al. (2016) observed a significant reduction in MDA.

Conclusion

Chitosan and lovastatin present a similar hypolipidemic activity and significantly reduced body fat levels and increased antioxidant levels in the blood serum of male rabbits.

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