EFFECTS OF CHITOSAN AND SALVADORA PERSICA ON BLOOD LIPIDS IN THE WISTAR RAT

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SUMMARY: Hypercholesterolemia is a metabolic disorder that ultimately results in arterial sclerosis and complications like hypertension and coronary arterial diseases. Various drugs have been used for treatment of this condition and many studies are underway to be used in the future. Chitosan and Salvadora Persica are two such drugs. Chitosan is produced by deacetylation of chitin which is present mainly in the exoskeleton of crustaceans. The aim of this in vitro study was to study the effects of these two drugs on blood lipid levels.

In this Interventional Laboratory Trial, 30 mature vistar rats weighing 200-250 grams were selected and after a period of two weeks of adaptation to the surroundings, they were allotted randomly to 6 groups. The rats were then fed for a period of 15 days with normal or fatty diet, with or without the drugs. Chitosan in pure powder form and persica in the form of hydro alcoholic, Salvadora persica stem extract were added to the diet of the respective study groups. At the end of this period, blood samples were taken in order to measure cholesterol, triglyceride, and HDL and LDL levels. Data were analyzed statistically using SPSS software program and Scheffe, ANOVA and Descriptive statistical tests.

Both chitosan and persica decreased cholesterol and LDL levels in the groups ingesting fatty diet ($P < 0.05$) and the mean reduction was not statistically different for the two drugs ($P > 0.05$). The two drugs had no effect on triglyceride and HDL levels ($P > 0.05$). Both chitosan and persica had no effect on blood lipid levels of subjects on normal diet whose cholesterol levels were normal ($P > 0.05$).

Persica and chitosan have similar effects on reduction of cholesterol and LDL levels in cases of hypercholesterolemia, but have no effect on triglyceride and HDL levels.

Key Words: Chitosan, salvadora persica, blood lipids, hypercholesterolemia.

INTRODUCTION

High blood cholesterol levels can result in atherosclerosis, growth of atheromatous lipid plaques on the intimal layer of blood vessels (1). Since most cholesterol in the body is used for production of bile acids and 95% of the bile is reabsorbed from the intestinal lumen, a great deal of attention has been focused on agents such as chitin, chitosan and Salvadora Persica which prevent bile absorption from the intestine and thus lead to stimulation of new bile production from hepatic cholesterol (2). Most studies report a reduction in the cho-

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Figure 1: Mean blood cholesterol levles (mg/dL) in the study groups.

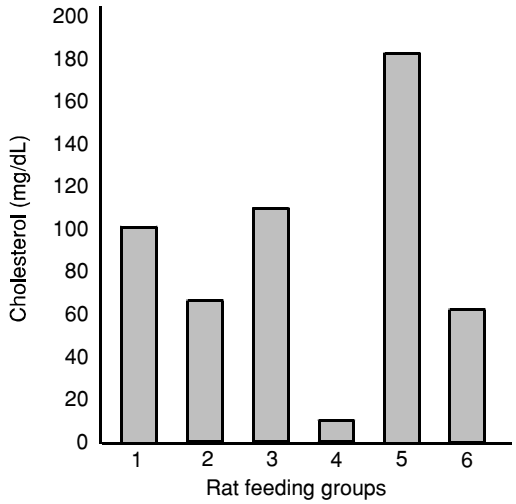
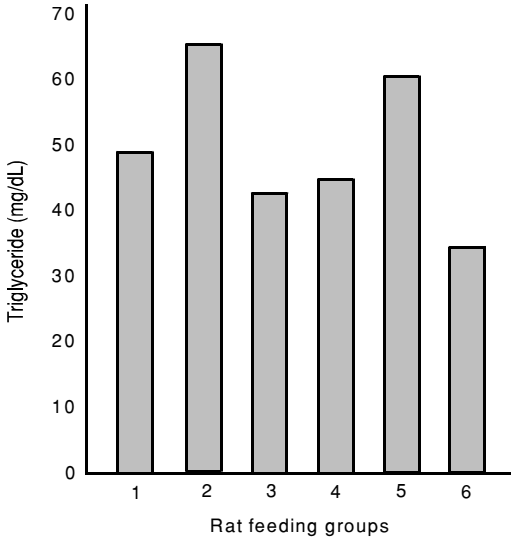


Figure 2: Mean blood triglyceride levles (mg/dL) in the study groups.



1: Fatty diet with persica extract
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6: Normal diet

lesterol level in rats after administering chitosan and persica, but the reduction level is different according to the doses of the drugs administered and duration of treatment.

Yasuhiko Fukada and co-workers reported that use of 20.1% chitosan (45.2mg/dl - 36.1 mg/dl) reduces blood cholesterol levels (3). Grondin and Lehoux studied the effect of chitosan on hepatic functions. According to the results of their study, addition of 5% chitosan to diet of rats resulted in 54% reduction of plasma cholesterol levels and 64% reduction in hepatic cholesterol. Similarly, chitosan in higher amounts results in greater reduction of plasma cholesterol levels (4). Ormrod *et al.* studied the effectiveness of chitosan in prevention of hypercholesterolemia and atherosclerosis in rats with apolipoprotein E deficiency and found 64% reduction in blood cholesterol level. There was also a 42-50% reduction in formation of atherosclerotic plaques in the study group (5). Gallaher *et al.* studied the complimentary effects of glucomanan fibers and chitosan in reduction of plasma cholesterol and increase in excretion of cholesterol in heavy weight individuals with normal cholesterol levels. According to their results, there was a significant reduction in LDL; HDL and blood cholesterol levels during the final stage, but the serum triglyceride levels did not alter significantly (6).

The aim of this study was to evaluate and compare the effects of chitosan and salvadora persica on blood lipid levels in rats.

MATERIALS AND METHODS

A controlled Interventional experimental study was conducted on thirty white wistar male adult rats obtained from the animal research center. The rats were about 12 weeks old, weighing between 200 and 250 grams and had not been under any prior investigations. The rats were divided into six groups of 5 rats each. All rats received rat pellets and they were named according to the fatty diet and treatment given. Thus the six groups were as follow: group 1 as persica/fatty group receiving fatty diet with persica extract, group 2 as persica/blank group receiving persica extract with no fat, group 3 as chitosan/fatty group receiving fatty diet with chitosan powder, group 4 as chitosan/blank group receiving chitosan powder with no fat, group 5 as control/fatty group receiving fatty diet with no treatment and group 6 as control/blank group receiving no fatty diet and no treatment.

Chitosan was procured from an Australian firm, Spring Leaf in the form of packets containing ninety Chitosan capsules (Figure 1). Persica extract was made by the hydroalcoholic method of extraction from dried salvadora persica stem powder (7) (Figure 2).

Table 1: Six types of Diet and weight of each constituent in relation to the total weight (P = 0.000).

Diet Type	Pellets (%)	Chitosan (%)	Persica (%)	Cholesterol (%)	Cholic Acid (%)	Oil (%)
Fatty diet with Chitosan	85.5	7	-	2	0.5	5
Fatty diet with Persica	85.5	-7	2	-	0.5	5
Normal diet with Chitosan	93	7	-	-	-	-
Normal diet with Persica	93	-	7	-	-	-
Fatty diet	92.5	-	-	2	0.5	5
Normal diet	100	-	-	-	-	-

Thus all 500 grams of persica stem powder was immersed in 2 liters of 80% ethyl alcohol solution and after 48 hours, the solution was filtered.

Determination of the amount of dry persica extract in the solution: At this stage, using a vacuum pump, 0.5 ml of the concentrate was dropped on a previously numbered and weighed filter paper delicately taking care not to puncture the paper. A digital scale with an accuracy of one thousandth of a gram (German made; Mettler AE200) was used. After the paper was completely dry, it was weighed again. In order to reduce errors, three papers were used each time. The mean difference in the weight of the papers before and after dropping the concentrate was defined as dry weight of persica extract in 0.5 ml of solution.

After evaporation of most of the alcohol in the solution over 48 hours at room temperature, it was transferred for 6 hours to an oven heated to 50 degrees centigrade (Iranian made, Bahman Co.) (Figure 2). Thus, from every 500 grams of dried salvadora persica stem powder, a concentrate solution containing 36.5 grams of dry persica extract was obtained.

In the study, 6 types of meals were prepared (Table 1).

Normal diet: Rat pellets were procured from the Pars animal feeds company. Diet containing 7 grams persica powder per 1000 grams of food was prepared by adding amount of concentrate solution containing 7 grams of dry persica extract to powdered rat pellets. Diet containing 7 grams chitosan powder per 1000 grams of food was prepared by first mixing chitosan powder with powdered pellets which was turned into dough using distilled water and then formed into pellets using 5 ml syringes.

Fatty Diet: In order to make the food fatty, 0.5% cholic acid (Merck Co. product; code No. 3671), 2% cholesterol (Merck Co. product; code No. 3672) and 5% Soya oil was added. The procedure was as follows: After heating 5 grams of Soya oil to 70 degrees Celsius, 2 grams cholesterol and 0.5 grams cholic acid was dissolved in it. The mixture was then poured over 92.5 grams of dry food and mixed thoroughly till a white jelly layer was formed on the surface of the pellets.

The rats were maintained in polycarbonate cages with anti rust steel doors at room temperature and normal light conditions. Two weeks was considered as the time required for the rats to adapt to the new surroundings and during this period they were fed with rat pellets. They had easy access to both drinking and tap water via a special polycarbonate vessel with an anti rust door. Each group was fed with the respective diet for a period of two weeks. For the measurement of blood levels of cholesterol, LDL, HDL and triglycerides (Figures 1, 2, 3 and 4), 2 ml of blood was drawn from the heart. At the end of two weeks, the rats were starved for one night. (Allowed water) (4). The rats were then placed in glass compartments containing cotton soaked with ether (diethyl ether 97%) in order to anesthetize them (8). The required amount of blood was drawn from their heart using 5 ml syringes (23 gauge needles) without killing the rats (9). The rats were held stable with the hands in the supine position and needle was inserted below the diaphragm at an angle of 20 - 30 degrees from the horizontal in the heart and 2 - 5 ml of blood was drawn (9). After clotting, the samples were centrifuged at a speed of 3000 rpm for 10 minutes (7). The serum samples were sent to the central laboratory for investigations. The laboratory is accredited both externally and internally.

Statistical Analysis

Data were analyzed by SPSS software program and statistical counseling. Initially, the mean and deviation results were determined by Descriptive Statistics tests and then ANOVA test was used to determine the differences. Ultimately, Sheffe test was used to compare the results of each group with the other groups.

Figure 3: Mean blood HDL levles (mg/dL) in the study groups.

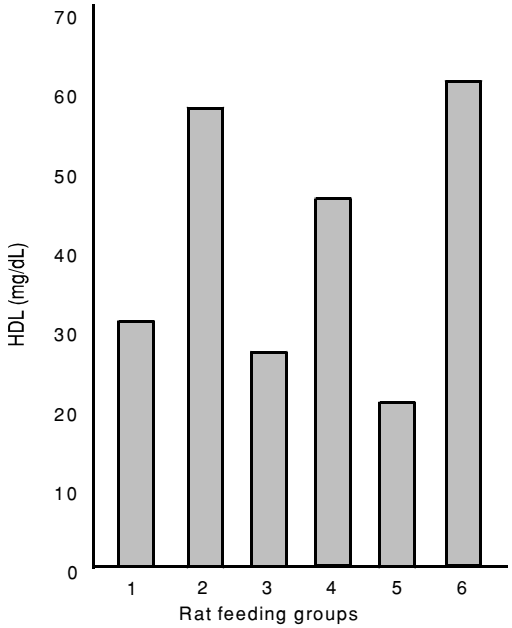

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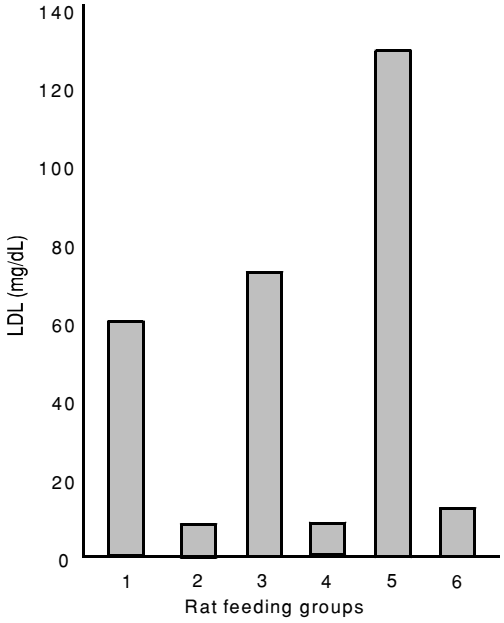
Figure 4: Mean blood LDL levles (mg/dL) in the study groups.



1: Fatty diet with persica extract
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RESULTS

The mean and standard deviation of blood cholesterol values of all 6 groups are shown in Table 2. The mean cholesterol level in the Control/Fatty group was 34.2% more than the Control/Blank group (P = 0.000). The cholesterol levels in the Persica/Fatty group were 35.5% less than the Control/Fatty group (P = 0.000). The cholesterol levels in the Chitosan/Fatty group were 29.8% less than the Control/Fatty group (P = 0.034). Both the mean cholesterol levels in the Persica/Blank and Chitosan/Blank groups were not statistically different from the Control/Blank group (P = 0.975 and P = 0.993). The mean cholesterol levels in the Persica/Fatty and Chitosan/Fatty groups were not statistically different (P = 0.990).

The mean and standard deviation of blood triglyceride levels of 6 groups is presented in Table 3. The triglyceride levels in the Control/Fatty group were 56.9% more than the Control/Blank group (P = 0.001). There was no significant change in the triglyceride levels of Persica/Fatty and Chitosan/Fatty groups (P = 0.888 and P = 0.409). The mean triglyceride levels in the Persica/Fatty and Persica/Blank groups with the Control/Fatty group were not significantly different (P = 0.996 and P = 0.130).

The mean and standard deviation of HDL levels of 6 groups is presented in Table 4. The mean HDL levels in the Persica/Fatty and Persica/Blank groups with the Control/Fatty group were not significantly different. (P = 0.876 and P = 0.513).

The mean and standard deviation of LDL levels of 6 groups is presented in Table 5. The LDL levels in the Persica/Fatty group were 53.3% less than the Control/Fatty group (P = 0.003). Similarly, the LDL levels in the Chitosan/Fatty group were 44% less than the Control/Fatty group (P = 0.020). Both the mean LDL levels in the Persica/Blank and Chitosan/Blank groups were not statistically different from the Control/Blank group (P = 0.996 in both cases).

Table 2: Mean blood cholesterol levels (mg/dL) in the study groups (P = 0.000).

Group	Diet Type	Mean Cholesterol Level	Standard Deviation (SD)	Minimum level (MIN)	Maximum Level (MAX)
1	Fatty diet with persica extract	102.00	22.00	76.00	137.00
2	Non fatty diet with persica extract	67.80	13.10	55.00	86.00
3	Fatty diet with chitosan extract	111.00	28.56	82.00	154.00
4	Non fatty diet with chitosan extract	11.40	6.18	45.00	60.00
5	Fatty diet	185.20	26.05	128.00	200.00
6	Normal diet	63.60	10.33	52.00	78.00

Table 3: Mean blood triglyceride levels (mg/dL) in the study groups (P = 0.000).

Group	Diet Type	Mean Triglyceride Level	Standard Deviation (SD)	Minimum level (MIN)	Maximum Level (MAX)
1	Fatty diet with persica extract	49.00	9.27	35.00	60.00
2	Non fatty diet with persica extract	65.60	16.00	47.00	88.00
3	Fatty diet with chitosan extract	42.60	8.87	32.00	52.00
4	Non fatty diet with chitosan extract	44.80	7.98	32.00	51.00
5	Fatty diet	60.40	9.34	57.00	81.00
6	Normal diet	34.40	5.85	26.00	40.00

DISCUSSION

In the present study the effects of Chitosan and Salvadora Persica were studied on normal as well as raised blood lipid levels in rats. According to the results of the study certain amount of chitosan had a direct relationship with decrease in cholesterol levels, but high doses of about 10% of the diet weight or more decreased growth of the animal as well (4, 10). Results also showed that in cases where cholesterol levels are above normal, persica extract can lower cholesterol levels by 35.5% in 15 days. In the study conducted by Galati and co-workers, however, the cholesterol levels decreased by 10% in 15 days and 23% at the end of 30 days (11). The difference in results between the present study and the Galati study could be due to the higher doses of the drugs and the lower time period of fatty diet administration in the present study (11).

The administration of 7% chitosan along with a fatty diet resulted in 29.8% reduction in cholesterol levels which is similar to other studies (4-6, 12-15).

In the study by Meng-Tsan Chiang and co-workers, prescription of 7.5% chitosan along with a fatty diet containing 1% cholesterol and 0.2% cholic acid decreased cholesterol levels by 57% (14). This high rate could be due to the young age of the rats and the period of high density growth which makes the animal more sensitive to diet change (2).

In addition, it could be argued that the lower levels of cholesterol and cholic acid in the diet and longer duration of study were important factors resulting in differences.

In the study by Lehoux and Grondin, addition of chitosan to a fatty diet comprising of 1% cholesterol and 0.2% cholic acid over three weeks resulted in a 54%

Table 4: Mean blood HDL levels (mg/dL) in the study groups (P = 0.000).

Group	Diet Type	Mean HDL Levell	Standard Deviation (SD)	Minimum level (MIN)	Maximum Level (MAX)
1	Fatty diet with persica extract	31.60	9.98	19.00	46.00
2	Non fatty diet with persica extract	58.40	4.66	52.00	63.00
3	Fatty diet with chitosan extract	27.80	5.97	22.00	37.00
4	Non fatty diet with chitosan extract	46.60	8.84	37.00	61.00
5	Fatty diet	21.20	5.67	17.00	31.00
6	Normal diet	61.80	10.15	49.00	75.00

Table 5: Mean Blood LDL levels (mg/dL) in the study groups (P = 0.000).

Group	Diet Type	Mean LDL Level	Standard Deviation (SD)	Minimum level (MIN)	Maximum Level (MAX)
1	Fatty diet with persica extract	60.60	32.53	20.00	111.00
2	Non fatty diet with persica extract	8.60	6.58	1.00	18.00
3	Fatty diet with chitosan extract	72.80	32.08	44.00	122.00
4	Non fatty diet with chitosan extract	8.40	4.15	1.00	11.00
5	Fatty diet	130.00	27.99	100.00	175.00
6	Normal diet	12.20	2.48	10.00	16.00

reduction in blood cholesterol levels. It seems that the longer time period of study, lower levels of cholic acid and cholesterol in the diet, and use of Long-Evans rats as subjects were responsible for greater reduction of cholesterol levels in that study (4).

The results of the present study show that both chitosan and persica have no effect on blood triglyceride levels. Similarly, Galati and co-workers concluded that persica has no effect on blood triglyceride levels (11). Meng-Tsan Chiang and co-workers also concluded that chitosan has no effect on blood triglyceride levels (14).

The effects of chitosan and persica on HDL levels were similar to the results presented.

In the studies by Meng-Tsan Chiang and Lehoux, Grondin, and Galati (4, 11, 14).

In the present study, persica resulted in decrease in LDL levels by 53.3%, while in the study by Galati, LDL levels decreased by 18% in the first 15 days and 38% after 30 days (11). The difference could be due to higher doses of the drug.

In the present study, chitosan resulted in decrease in LDL levels by 44% which is similar to other studies (4-6, 9, 12, 13). The levels of LDL decreased by 76% in the study by Meng-Tsan Chiang and co-workers (14). This greater decrease could be due to the fact that the rats were younger, amount of cholesterol and cholic acid in the diet was lower and the time period of study was longer (14).

The study by Ormrod and co-workers points out to the reduction in the formation of atherosclerotic plaques in the walls of big vessels by chitosan and its effectiveness in decreasing LDL levels; one of the main causes of atherosclerosis (5).

Meng-Tsan Chiang and co-workers postulated that decrease in plasma VLDL levels due to interference by chitosan in digestion and absorption of lipids and increase in hepatic LDL receptors resulted in lowering of blood LDL levels (14). Galati and co-workers postulated a similar theory for the effects of persica on LDL levels (11).

Administration of persica in rats on a non fatty diet had no statistically significant effect on any of the mentioned indicators ($P > 0.05$). Similarly, chitosan also had no statistically significant effect on cholesterol, triglyceride, and HDL or LDL levels ($P > 0.05$). Thus it could be concluded that both drugs have no effect on normal cholesterol levels ($P > 0.05$). Fukada and co-workers, studied chitosan under no fatty diet, and it resulted in a 20% reduction in normal cholesterol levels (3).

One possible explanation for ineffectiveness of the drugs in rats on normal diet is that bile salts play an important role by breaking up large droplets of fat during the process of digestion (16). Lipids present in food are themselves stimulating factors for secretion of bile from the liver (2). It is postulated that chitosan adheres to the bile salts resulting in their excretion from the body via the intestines. Thus, ingestion of fatty foods along with chitosan results in greater excretion of bile salts from the liver and therefore more cholesterol is needed for production and replacement of bile salts (17). Persica also acts in a similar manner increasing the use of cholesterol in the liver in order to replace lost bile salts (11).

The present study showed that the effectiveness of persica in lowering cholesterol and LDL levels in hypercholesterolemic rats was similar to the effects of chitosan when used in similar quantities. None of the studies to date have compared the effectiveness of two drugs and the study is unique in this respect.

CONCLUSION

Persica and chitosan lower cholesterol and LDL levels in hypercholesterolemic rats, but both drugs have no effect on triglyceride and HDL levels.

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