A RABBIT MODEL FOR STUDYING HYPOCHOLESTEROLEMIC EFFECT OF DRUGS AND HYPOCHOLESTEROLEMIC EFFECT OF EXTRACTS OF GARLIC (*ALLIUM SATIVUM*)

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ABSTRACT

An experimental model of hypercholesterolemic rabbits suitable for studying the hypocholesterolemic effect of compounds was developed. Rabbits were made hypercholesterolemic by oral administration of cholesterol (100 mg/kg body weight/day) suspended in ground nut oil by gastric intubation (Ryle's tube). Cholesterol can be given to rabbits from 10 days to 6 months depending on the degree of hypercholesterolemia required and duration of study of hypocholesterolemic effect. In one month cholesterol feeding experiment, the serum cholesterol level in normal controls (not given cholesterol) was 67 ± 11.3 mg/dl and in cholesterol fed animals 191.3 ± 70.6 mg/dl. In 2 months experiment, besides hypercholesterolemia, abnormal serum lipid profile and increase in lipid content in liver, heart and aorta were also seen. To such animals when water and methanol extracts of garlic were given along with cholesterol, there was significant decrease in serum cholesterol level. The advantage of this method over the method in which cholesterol is mixed with diet to induce hypercholesterolemia is that exactly same and calculated amount of cholesterol can be given for each animal. This minimises the variations in serum cholesterol levels in different animals.

KEY WORDS: Animal model, Hypercholesterolemia, Hypocholesterolemic effect, garlic, *Allium sativum*.

INTRODUCTION

In the most commonly used method for testing the hypocholesterolemic effect of unknown compounds, the experimental animals are made hypercholesterolemic by feeding them with cholesterol mixed with the diet (1,2). This method suffers from one drawback that the amount of diet and consequently the amount of cholesterol taken by each animal is not uniform. This in turn results in wide variations in serum cholesterol levels reflected in high standard deviation (SD) in mean $\pm$ SD values. This makes the interpretation of results difficult.

In view of the high incidence of atherosclerosis all over the world, the need for the development of newer hypocholesterolemic drugs tested in appropriate experimental animals for use in such diseases needs to be hardly stressed. As part of our work to screen some plants or drugs for their hypocholesterolemic effect, we faced the above mentioned practical difficulties, when we made rabbits hypercholesterolemic by feeding them with cholesterol in diet.

We therefore developed an alternate method for inducing hypercholesterolemia in rabbits. The results are reported here. The usefulness of model was tested with garlic extracts.

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MATERIALS AND METHODS

Chemicals: Cholesterol (extrapure) for feeding purpose was purchased from Loba Chemie, Mumbai.

Animals: Rabbits weighing about 1 kg were purchased from the local breeders and maintained on Hindustan Lever pellet diet for rabbits.

Inducing hypercholesterolemia

Cholesterol was suspended in groundnut oil. Calculated amount (100 mg/kg body wt.) was given to the rabbits by gastric intubation using Ryle's tube. To the controls same volume of groundnut oil was given.

Cholesterol estimation: Cholesterol was estimated with kits from Ortho Diagnostics, Mumbai.

RESULTS AND DISCUSSION

In order to determine the optimum dose of cholesterol for inducing adequate hypercholesterolemia, four groups of 5 rabbits each were used. Group 1, without cholesterol, served as controls and received groundnut oil. Animals of groups 2, 3 and 4 received 100, 200 and 500 mg/kg body wt/day orally for 7 days as described under methods. After 7 days the serum cholesterol levels were found to be 69.0 ± 5 mg/dl in control group and 142 ± 40 , 209 ± 81 and 557 ± 320 mg/dl respectively in groups 2, 3 and 4. In humans, one does not normally deal with serum cholesterol values more than 1.5 to 2 times the upper limit of normal range of about 250 mg/dl. In fact, feeding of 500 mg/kg cholesterol for a slightly longer time of 15 days resulted in serum cholesterol levels of 1000 mg/dl or above, which are unrealistic, too high and 15-20 times the normal values for rabbits. These serum cholesterol levels are very high perhaps for a drug to show hypocholesterolemic effect. So doses of 100 and 200 mg/kg were considered for subsequent studies to choose the optimum dose.

Assessment of hypocholesterolemic effect

We used extracts of garlic, well known as a cholesterol lowering (1-4) and as an antibacterial including antitubercular agent (5,6) both in animals and in humans. Water and methanol extracts (100g extracted with 50 ml thrice at 4°C) were used for their hypocholesterolemic effect. In the case of methanol extract, the solvent was removed

by evaporation under reduced pressure.

We tried two methods: (i) administration of cholesterol and drug simultaneously to hypercholesterolemic rabbits (ii) administration of drug to hypercholesterolemic rabbits without cholesterol.

Effect of garlic extract when administered simultaneously along with cholesterol to hypercholesterolemic rabbits

Rabbits were made hypercholesterolemic by feeding 200 mg/kg daily once for 10 days. The fasting cholesterol levels were estimated to ensure hypercholesterolemia in rabbits. These were shown as initial values in table 1. Then the rabbits in group 2 were given orally water extract (equivalent to 10 g of fresh garlic) for one week along with cholesterol. Group 1 rabbits did not receive the drug but were given cholesterol. Serum cholesterol values at the end of the experiment were shown as final values.

Table 1. Effect of water extract of garlic on serum cholesterol levels in hypercholesterolemic rabbits.

Group	Serum (total) cholesterol (mg/dl)			
	Basal	Initial	Final	% Rise*
1. Control	41.3 ± 12	461 ± 228	635 ± 231	37.5
2. Water extract (garlic)	53.3 ± 12	520 ± 281	626 ± 372	20.5

Values represent mean $\pm$ SD of 5 animals in each group, * % rise in final values when compared to initial values.

The results in Table 1, show that in the control animals receiving cholesterol only without the drug, the rise in serum cholesterol was 37.5% over the initial value. In the rabbits treated with the water extract of garlic, the rise was only 20.5%. The difference was significant ($P < 0.05$). This shows that this animal model can assess hypocholesterolemic effect and water extract of garlic has hypocholesterolemic effect.

Administration of drug only to hypercholesterolemic rabbits without simultaneous administration of cholesterol

Basal serum cholesterol levels of 2 groups

of rabbits (5 each) were determined. They were made hypercholesterolemic by feeding 200 mg/kg cholesterol daily once for one week. The serum cholesterol levels (fasting) were estimated to ensure hypercholesterolemia. These were initial values. Then the cholesterol administration was stopped to both the groups. Animals in group 2 received methanol extract of garlic (equivalent to 10g of fresh garlic) orally for one week, while control group 1 animals were given control solvent treated in a similar way (removal of solvent under reduced pressure). Serum cholesterol values at the end of the experiment were shown as final values.

Table 2. Effect of methanol extract of garlic on serum cholesterol levels in hypercholesterolemic rabbits

Group	Serum total cholesterol (mg/dl)			
	Basal	Initial	Final	% Fall*
1. Control	41.3±12	253.3±128	198.3±88	21.7
2. Methanol extract (garlic)	53.3±12	466.6±233	223.3±120	52.1

Values represent mean± standard deviation in 5 animals of each group* % fall final values when compared to initial values.

It is obvious from the results in table 2 that the control animals became hypercholesterolemic in one week. But after cholesterol administration was discontinued, the serum cholesterol levels started falling down as is anticipated. After one week without cholesterol, the fall in serum cholesterol level was 21.7%. But in the garlic treated animals the fall in serum cholesterol level was much higher (52.1%) and the difference was highly significant ($P<0.01$). The design of this experiment is similar to the situation in humans with hypercholesterolemia who will be advised to stop eating cholesterol and other fat containing food. Treatment would be given by the physician if the situation demands. In spite of this advantage, since there is fall in control animals also, the hypocholesterolemic effect of unknown compound can be detected only if its activity is high. It would be difficult to interpret results with slight activity. So we preferred the model in which the drug and

cholesterol are given simultaneously.

Effect of feeding 100mg/kg/day of cholesterol for making the rabbits hypercholesterolemic

It was felt that the values of serum cholesterol in rabbits made hypercholesterolemic by feeding 200mg/kg cholesterol daily once for 7-10 days which were 400-500 mg/dl are very high. These are 8-10 times the normal values of rabbits. Sometimes the experiments are to be carried out for longer periods of 1-2 months or more. In such cases the serum cholesterol levels would be still higher. Therefore a lower dose of 100mg/kg/day of cholesterol feeding was considered to be useful. The effect of feeding cholesterol 100 mg/kg/day for 1 and 2 months on serum cholesterol levels are shown in table 3.

Table 3. Effect of feeding cholesterol 100 mg/kg/day for one and two months daily on serum cholesterol levels

Group	Serum total cholesterol (mg/dl) after cholesterol feeding for			
	one month		two months	
	initial	final	initial	final
1. Normal	73.0±7	67±11	55.0±20	55.6±14
2. cholesterol fed	63.6±27	191±70	57.8±11	172.6±61

Values represent mean± standard deviation in 5 rabbits in each group.

The serum cholesterol levels of rabbits fed cholesterol 100 mg/kg daily once for one month were 191 ± 70 mg/dl which are about 3 times higher than the normal values. These levels are suitable for testing for hypocholesterolemic effect of drugs. Garlic extracts lowered the cholesterol levels (results not shown). These hypercholesterolemic animals, if untreated, showed besides hypercholesterolemia, abnormalities in other parameters of serum lipid profile and increase in levels of lipids in liver, heart and aorta. This model gave good results when it was used for testing the hypocholesterolemic effect of other plant extracts (unpublished data with banyan tree bark and fenugreek seeds).

Thus this model of hypercholesterolemic rabbits has been tested adequately with plant extracts and purified compounds with good results. The results also show that 100 mg/kg of cholesterol daily once can be given for periods ranging from 7 days to 2 months. We have given even for 6 months (unpublished data).

In conclusion, it can be stated that this method of making rabbits hypercholesterolemic by feeding cholesterol 100 mg/kg daily once by gastric intubation (Ryle's tube) has many advantages over the method of giving cholesterol mixed with the diet. Exactly same and calculated amount of cholesterol suspended in groundnut oil

or other suitable vehicle can be given to each animal. With a little practice, cholesterol can be administered by Ryle's tube without any damage or even irritation to the alimentary tract of the rabbits. Although individual variations in serum cholesterol levels can not be eliminated, the variation is less than with the other method, in which all the animals do not consume the same amount of diet and hence cholesterol. The animals showed abnormalities in serum lipid profile, tissue lipids and histopathology of tissues (results not shown here). Plant extracts and compounds could bring about reduction in serum cholesterol levels and other lipid parameters.

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