

Comparative study of the effect of atorvastatin and garlic extract in experimentally induced hypercholesterolemia in rabbits

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ABSTRACT

Background: The abundant resource of drugs and its beneficial properties are hidden in our natural and Indigenous sources, which are under constant evaluation by man. Cholesterol lowering ability of ethanol extract of garlic was evaluated in comparison to atorvastatin, the most frequently used lipid lowering agent in rabbits.

Methods: Hypercholesterolemia was induced to the animals with cholesterol powder (50mg/kg) for the study duration (16 weeks). At the end of 4 weeks, they were randomly selected and divided into 3 groups (n=6). Group II received Cholesterol + atorvastatin 10 mg daily; Group III received Cholesterol + 0.1g garlic extract kg b. w. daily while Group I continued with cholesterol powder (to serve as control) for the rest study period. Serum cholesterol, LDL, HDL and Triglycerides were estimated using the enzymatic method at 0, 4, 8, 12, 16 weeks in all the groups. The results were tabulated and analyzed statistically using one way ANOVA test.

Results: The results indicate that both atorvastatin and garlic extract have a definite role in retarding the rate of weight gain as a consequence to high cholesterol diet in rabbits. Also, there is fewer rises in all the lipid parameters in both the treatment groups when compared to the control group.

Conclusion: Though atorvastatin is definitely more effective in reducing the lipid parameters but it also significantly lowers HDL where as Garlic shows promising results when compared to placebo and also has a favourable effect on HDL. Garlic can be recommended as a dietary supplement for long term use without toxic effects for its wide range of medicinal properties in general and therapeutic potential in patients with CAD in particular.

Keywords: Hypercholesterolemia, Garlic, Atorvastatin

INTRODUCTION

The consistent observation that human atherosclerosis is prominent in clinical states accompanied by hypercholesterolemia have strongly implicated cholesterol as an important etiological agent in the development of atherosclerosis (Brodia et al 1978)¹ and associated conditions like coronary artery disease, ischemic cerebrovascular diseases etc. A triangular relationship between high fat diet, blood cholesterol-lipoprotein levels and coronary heart disease has been established since a long time (WHO 1982).² Among the lipoproteins, LDL cholesterol has been found to be directly associated with CHD (Gordon et al, 1977).³

Many drugs are used in the treatment of hypercholesterolemia of which the statins are most frequently prescribed. Statin-mediated lowering of low-density lipoprotein cholesterol (LDL -C) is regarded as the foundation of lipid-modifying therapy. However, this has failed to reduce cardiovascular event rates by more than about 20%-40% relative to placebo (Kastelein et al, 2005)⁴ indicating the need for therapeutic intervention against other lipid targets as well as non-lipid risk factors. Moreover, the potential side effects limit long term use of these drugs. Lifestyle modification and pharmacological measures must be taken together to achieve the target. In recent years there is ongoing search for natural substances that can combat hypercholesterolemia. Folic

acid, L-carbinitine, red wine, green tea, and garlic have shown promising results. Of these, garlic (*Allium sativum*) gets particular attention in Modern Medicine because it has high concentration of thiosulfonates, including allicin that appears to be the active principle. This and other related substances inhibit HMG Co-A reductase involved in cholesterol biosynthesis and prevents significant rise of serum cholesterol and also increases fibrinolytic activity in patients of CHD.³ The present study was undertaken to evaluate the cholesterol lowering ability of ethanol extract of garlic in comparison to atorvastatin, the most frequently used lipid lowering agent in experimental animal model (rabbit).

METHODS

Animals

Rabbit has been used as experimental animal in studies related to hypercholesterolemia and subsequent atherosclerosis since 1913 (Anitschkow et al, 1913).⁵ Healthy male rabbits weighing between 1- 1.25 kg, 6 months to 1 year of age were used for this experiment. The rabbits were kept in individual polysulfone cage with wire mesh bottom. They received normal diet containing Bengal gram, green grass, one tea spoonful maize flower and drinking water at libitum. The temperature was maintained at 22-26°C while the relative humidity was 50-60%. The experiment was conducted in the Department of Pharmacology, MGM Medical College & Hospitals, Jamshedpur in collaboration with Department of Pharmacology, Medical College, Kolkata. The experiments were performed following approval by the Institutional Animal Ethics Committee.

Drugs and Chemicals

Cholesterol powder
Olive oil
Atorvastatin-10mg (Lupin)
Ethanol extract of garlic

Preparation of Ethanol extract of Garlic

300 gm of wet minced garlic was kept in a two liter flask containing one liter pure ethanol for 48 hours. During this period the flask was shaken occasionally to ensure thorough extraction. Then the solvent (ethanol) was removed on a rotatory evaporator at a temperature of

40°C under vacuum and oily extract remaining in the flask was made moisture free and kept in a dessicator at 4°C. The ethanolic extract yielded 46.9 mg oil per gram of raw garlic. The rabbits were given 0.1g extract per kg body weight per day in 1 ml olive oil.

Procedure

All the rabbits (n=18) received normal diet for pre experimental period of 4 weeks for adaptation. At the end of this period, animals were weighed, and fasting blood samples were taken from the marginal ear vein for estimation of serum lipids.

Total experimental period of study was 16 weeks. Hypercholesterolemia was induced to the animals by feeding them with cholesterol powder 50 mg /kg dissolved in 5 ml of olive oil daily for the whole duration of study (16 weeks). At the end of 4 weeks, they were randomly selected and divided into 3 groups (n=6). Group II received Cholesterol + atorvastatin 10 mg(10 mg tablets were powdered and mixed with 5ml of glycerine and fed with the help of a cannula to the animals) daily; Group III received Cholesterol + 0.1g garlic extract per kg body weight per day in 1 ml olive oil and Group I continued with cholesterol powder (to serve as control) for the rest study period of 12 weeks. After the end of study, all the drugs were withdrawn, and the rabbits were kept on normal diet for a convalescent period of six months before considering them for any other study.

Serum cholesterol, LDL, HDL and Triglycerides were estimated using the enzymatic method at 0, 4, 8, 12, 16 weeks in all the groups. The results were tabulated and analyzed statistically using one way ANOVA test.

RESULTS

There was a steady weight gain in the rabbits of all the groups as they were fed with cholesterol diet. The increase was less in Group II and Group III in comparison to Group I, although Group II & III were comparable (Table 1). There was a steady rise in all the lipid parameters in Group I, II and III but the rise was significantly less in treatment groups II & III. Comparing Group II and III, atorvastatin more effectively reduced the cholesterol, LDL, TG levels than garlic extract while the HDL level was more in Group III.

Table 1: Mean body weight (0, 4, 8, 12 & 16 weeks) of the groups I, II, III (n=6).

	weight in grams (mean± SD)				
	0	4	8	12	16
Group I	1150 ± 70.36	1204 ± 74.13	1281.6 ± 53.97	1323.3± 45.44	1365.8 ± 56.46
Group II	1137 ± 69.49	1152 ± 70.31	1176 ± 73.93	1204 ± 74.11	1226 ± 73.27
Group III	1141 ± 47.33	1157 ± 59.6	1186 ± 57.9	1219 ± 58.8	1243 ± 71.1

Table 2: Lipid profile for 16 weeks in experimentally produced hypercholesterolemia in rabbits.

Garlic pearls	Cholesterol	LDL	TG	HDL
Baseline	46.6 ± 3.08	26.01 ± 3.59	37.7 ± 4.8	11.6 ± 1.3
4weeks	89.8± 6.98	53.84 ± 2.6	37.7 ± 4.8	23.9 ± 2.9
8weeks	119.6±3.8	65.93±6.1	67.7 ± 4.1	32.1±4.8
12 weeks	135.9 ±4.8	78.4±4.5	77.2±3.9	39.3±5.1
16 weeks	148.1±5.4	91.9±3.9	89.22±5.19	41.4±3.2
P value	<0.0001	<0.0001	<0.0001	0.61

Table 3: Effects on the lipid profile of Atorvastatin 2 mg for 16 weeks in experimentally produced hypercholesterolemia in rabbits (n=6).

Ator 2mg + Chol powder	Cholesterol	LDL	TG	HDL
Baseline	47.5 ±3.08	25.08± 3.59	33.7 ± 4.8	12.6 ±1.3
4weeks	93.1± 6.98	48± 6.2	57.7±4.1	24.9 ±12.9
8weeks	110.6_± 4.11	57.12±3.4	65.88±5.4	30.3±3.8
12 weeks	122.3 ±4.9	63.7± 4.6	76.9±6.2	33.2±4.1
16 weeks	137.1± 3.1	68.6± 5.2	83.4± 4.33	37.9±6.3
P value	<0.0001	<0.0001	<0.0001	0.93

Table 4: Effects on the lipid profile of garlic pearls in experimentally produced hypercholesterolemia in rabbits (n=6).

Garlic pearls	Cholesterol	LDL	TG	HDL
Baseline	46.6 ± 3.08	26.01 ± 3.59	37.7 ± 4.8	11.6 ± 1.3
4weeks	89.8± 6.98	53.84 ± 2.6	67.7 ± 4.1	23.9 ± 2.9
8weeks	119.6±3.8	65.93±6.1	77.2±3.9	32.1±4.8
12 weeks	135.9 ±4.8	78.4±4.5	89.22±5.19	39.3±5.1
16 weeks	148.1±5.4	91.9±3.9	98.67±6.2	41.4±3.2
P value	<0.0001	<0.0001	<0.0001	0.61

DISCUSSION

Sedentary life style, urbanization, industrialization, alcohol, cigarette smoking etc increases the incidence of hypercholesterolemia and consequently atherosclerosis and CHD. So attempts are being made to find safe and effective drugs and dietary factors that would lower blood

cholesterol levels. All the currently available hypolipidaemic drugs have significant side effects and potential toxicities without defined benefits. They should be reserved for patients with familial hyperlipidemia or others at elevated risk of CHD. Even in these patients the drug should be discontinued after one to two months if the conc. of cholesterol is not reduced by at least an

additional 10% of the value achieved by dietary modification alone (Hunninghake et al, 1977).⁶

The statins (atorvastatin, rosuvastatin, lovastatin, simvastatin etc) are a class of hypolipidaemic drugs that act by inhibiting HMG Co-A reductase, the rate limiting enzyme in the mevalonate pathway of cholesterol biosynthesis. Inhibition of this enzyme in the liver stimulates LDL receptor resulting in an increased clearance of LDL from blood and decrease in cholesterol level by 30-50%. They also reduce the TG level but the rise of HDL level is less than fibrates and nicotinic acid (other hypolipidaemic drugs). The NCEP Guidelines, 2001 state that diet and lifestyle modification should be followed before starting statins or any other hypolipidaemic agents.⁷ Various studies like the Scandinavian Simvastatin survival study (4S) indicate that statins were useful in secondary prevention of cardiovascular diseases like Coronary artery disease, Myocardial infarction, stroke & peripheral artery disease and also in primary prevention only when the risk for cardiovascular disease was significantly high. However, the statins are not free from adverse effects. While some patients on statins report myalgia, muscle cramps and G.I. Symptoms, the major safety concern is myositis and rarely rhabdomyolysis (pathological breakdown of skeletal muscles) which leads to acute renal failure. The risk of myopathy and rhabdomyolysis increases in proportion to the plasma Statin concentration (Omar et al, 2001).⁹

Garlic has acquired a reputation in the folklore of many cultures over centuries as a formidable prophylactic and therapeutic medicinal agent. The medicinal properties of garlic are mentioned frequently in the old medicinal books (Warren, 1970).¹⁰ The Spanish Pharmacopeia of 1954 listed garlic among the drugs (Hindjo et al, 1968).¹¹

Garlic has attracted particular attention in modern medicine due to its wide range of medicinal properties for its antibacterial to anticancer effects (Srivastava et al, 1995 and Barness et al, 2002).^{12,13} It has hypolipidemic (Bordia et al, 1981)¹⁴ antithrombotic (Ali et al, 1995)¹⁵ and anti-atherosclerotic¹⁶ and hepatoprotective activity. It has high concentration of thiosulfinates that is responsible for reduction of risk factors of cardiovascular disease and cancer particularly of stomach and colon cancer. Bordia et al showed that garlic has a very significant protective action against hyperlipemia and blood coagulation changes.¹⁶ Sainani et al have found that the individuals who totally avoid taking garlic and onion have significantly high level of serum cholesterol, triglycerides, bola-lipoproteins and phospholipids, and those consuming small amounts of garlic and onion were better protected.¹⁸

The root bulb of garlic plant is used for medicinal purpose. Medicinally used garlic oil consists of diallyl (57%), allyl methyl (37%) & dimethyl (6%) mono to hexa sulphides (Koch et al, 1996).¹⁹ Many randomized clinical trials have

studied the effect of garlic on lipid levels. Results from two Meta analysis conducted in 1993 by Warshafsky et al²⁰ and in 1994 by Silagy et al²¹ on the effect of garlic on total cholesterol show a significant reduction in total cholesterol level (9-12% when compared with placebo). A more recent trial by Ackerman et al in 2001 is a placebo controlled trial using standardized dried garlic powder which shows significant reduction in total cholesterol levels (19.2mg/dl), LDL level (6.7mg/dl) triglyceride level (21.1mg/dl) in 8-12 weeks.²²

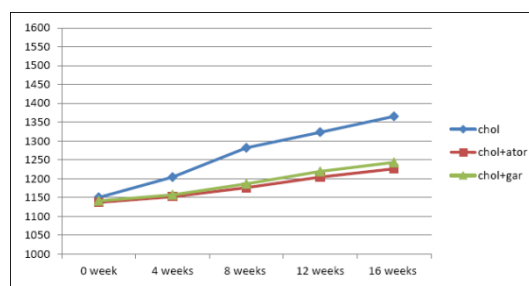


Figure 1: Showing the mean increase in body weight in the three groups.

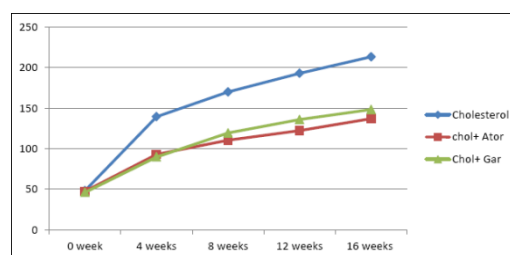


Figure 2: Showing the cholesterol level in the three groups for 16 weeks.

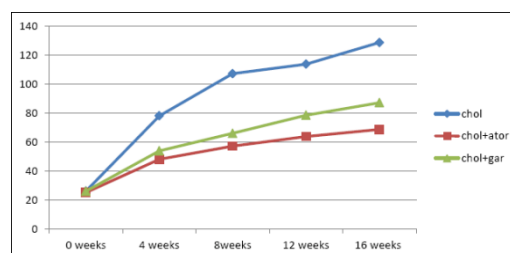


Figure 3: Showing the LDL value in the three groups for 16 weeks.

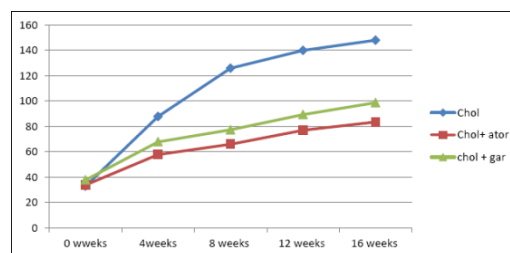


Figure 4: Showing the TG value in the three groups for 16 weeks.

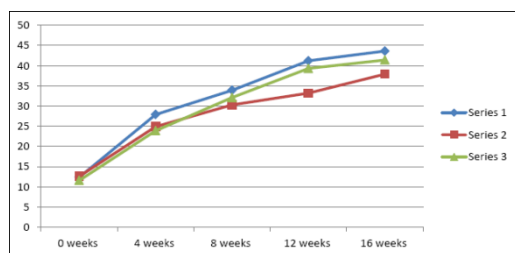


Figure 5: Showing the HDL value in the three groups for 16 weeks.

The results of the study indicate that both atorvastatin and garlic extract have a definite role in retarding the rate of weight gain as a consequence to high cholesterol diet in rabbits (Figure 1). Also, there is fewer rises in all the lipid parameters in both the treatment groups when compared to the control group. The results of the study corroborates with that of Carrol (1971) who have seen the plasma cholesterol levels and liver cholesterol biosynthesis in rabbits fed on commercial or semi synthetic diet with or without added fat or oils.²³ When compared to garlic extract, atorvastatin is definitely more effective in reducing the lipid parameters but it also significantly lowers the good cholesterol, i.e. HDL. Though Garlic is less efficacious than atorvastatin, it shows promising results when compared to placebo and also has a favourable effect on HDL. Garlic is commonly used as food additive and can be recommended as a dietary supplement for long term use without toxic effects. It has the added advantage of having wide range of medicinal properties in general and therapeutic potential in patients with CAD in particular. However further studies need to be done including clinical trials for further exploration of the medicinal prospects of garlic extract.

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