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Biochemical studies on induced hyperlipidemia and its amelioration using aged garlic extract in Wistar rats

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Abstract
The effect of aged garlic extract on different biochemical parameters was studied. The study included 72 Wistar albino rats, divided 12 in each group into six groups. Group I served as normal control group. To group II high fat diet was given as diet control. Rats in the group III were administered aged garlic extract at 200mg/kg bw. Group IV rats were administered aged garlic extract at 200mg/kg.bw with high fat diet. Rats in group V were administered atorvastatin at 15mg/kg.bw with high fat diet. The group VI rats with aged garlic extract and atorvastatin at half dose (100mg and 7.5mg/kg respectively) with high fat diet. The duration of the study was for 56 days and the blood samples were collected at 42nd and 56th day of study for evaluation. The high fat diet induced hyperlipidemia altered biochemical parameters; significant increase (P<0.05) total cholesterol, triglycerides, LDL-c, VLDL-c and reduction in the HDL-c. The serum enzymes like ALT, AST were also increased, but no change was observed in the serum levels of GGT, BUN and creatinine. The aged garlic extract treatment at the dosage used showed moderate improvements in the mean (±SE) values of lipid profile and serum enzymes, this suggested that the aged garlic extract could be used for improving the high fat diet induced serum biochemical abnormalities.

Keywords: high fat diet, hyperlipidemia, lipid profile, aged garlic extract

Introduction
Cardiovascular disease (CVD), which includes coronary heart disease, cerebrovascular disease, hypertension, heart failure and rheumatic heart disease, is a leading cause of death worldwide. Approximately 16.7 million or 29.2 per cent of total global deaths result from different forms of CVD every year [1].

The term hyperlipidemia refers to an increase in the concentration of lipids (i.e. triglycerides, cholesterol or both) in the blood (serum or plasma [2]. Hyperlipidemia is the current medical as well as social problem, it is associated with increased risk of atherosclerosis, which predisposes to ischemic heart disease and cerebrovascular disease [3].

All the modern drugs in use to lower blood cholesterol level, such as statins, fibrates, nicotinic acid and resins cause side effects. Therefore people are looking for safe alternatives of natural agents [4].

Garlic (*Allium sativum*) has an important dietary and medicinal role for centuries. It used as hypolipidemic, hypoglycaemic, anticoagulant, antihypertensive, antimicrobial, anticancer and antitumor, as an antidote for heavy metal poisoning and as an immunomodulator [5].

Garlic is rich source of organosulphur compounds, which are responsible for its flavour, aroma and potential health benefits. Extracts of fresh garlic that are aged over a prolonged period to produce aged garlic extract (AGE) contain antioxidant phytochemicals that prevent oxidant damage. These include unique water-soluble organosulfur compounds, lipid-soluble organosulfur components and flavonoids, notably allixin and selenium [6].

Atorvastatin, belongs to second generation of statins, is a synthetic reversible inhibitor of the microsomal enzyme HMG-CoA reductase. Among the available HMG-CoA reductase inhibitors, simvastatin and atorvastatin are the major two lipid lowering drugs used reduce the elevated lipid profiles in hyperlipidemic conditions. Statins, by inhibiting the synthesis of cholesterol, consistently reduce plasma low density lipoprotein (LDL)-cholesterol and total cholesterol levels and reduce cardiovascular morbidity and mortality in high risk patients with hyperlipidemia [7, 9].

The present study aimed to investigate the possible lipid lowering and enzyme profile modifying effects of aged garlic extract in induced hyperlipidemia in rats.

Materials and Methods

Drugs and chemicals: Aged garlic was procured from Wakunga of America Co. Ltd. USA. Atorvastatin API was procured from Microlabs India Pvt, Ltd. The cholesterol extra pure (Product number: 54181) from SRL chemical and cholic acid (Product number: C1129-500G) from Sigma chemicals were procured for preparation of high fat diet. All other chemicals and reagents used for the study were procured from local sources and were of analytical grade.

Animals: Normal adult Wistar albino rats weighing approximately 180-200 grams were procured from Biogen Laboratory Animal Facility (Reg.No-971/bc/06/CPCSEA) Bangalore, for conduct of the experiment for the study. They were maintained under standard laboratory conditions and fed with *ad libitum* standard commercial rat feed and high fat diet as per design and clean drinking water. The duration of experiment was for a period of 56 days and a prior permission was obtained from the Institutional Animal Ethics Committee (IAEC) for the conduct of the experiment.

High fat diet

To induce hyperlipidemia in the Group II, IV, V and VI rats, the diet prepared by adding 1 per cent of cholesterol, cholic acid 0.5 per cent and mixture of soya oil and vanaspathi ghee in the ratio of 2:3 @10 ml per kg of standard rat chow diet was fed *ad-libitum* throughout the study period of 56 days.

Experimental design

The rats were maintained under standard laboratory conditions for a period of 15 days for acclimatization in the experimental animal house. The rats were divided, based on the body weight, into six groups with twelve rats in each group. Group I served as normal control and gavaged with distilled water. To group II was high fat diet control fed *ad libitum*. Group III was aged garlic extract control given at 200mg/kg bw. Group IV aged garlic treatment group administered aged garlic extract at 200mg/kg.bw with high fat diet. Group V was atorvastatin treatment group administered atorvastatin at 15mg/kg.bw with high fat diet. The group VI was mixed treatment group administered aged garlic extract and atorvastatin at half dose (100mg and 7.5mg/kg respectively) with high fat diet.

To study the progressive effects of the treatments given to different groups, blood samples from different groups were collected on 42nd and 56th day of the experiment and analysed using Transasia EM 200 Automatic biochemical analyser and LDL and VLDL values were derived using Fridewald's equation.

Results and Discussion

The effect of aged garlic treatment in high fat diet induced hyperlipidemia was analysed for different serum biochemical parameters and results are presented in table 1 and 2.

In the present study, the high fat diet induced hyperlipidemia significantly ($P \leq 0.05$) increased the levels of total cholesterol, triglycerides, low density lipoproteins, very low density lipoproteins and significantly ($P \leq 0.05$) and reduced the levels of high density lipoproteins. The activity of alanine aminotransferase and aspartate aminotransferase were increased ($P \leq 0.05$) significantly but activity of gammaglutamyl transferase and levels of creatinine, blood urea nitrogen remained unaffected. High fat diet induces HMG CoA reductase enzyme due to availability of acetyl CoA stimulating cholesterogenesis [10]. Accelerated TG synthesis inhibits fatty acid metabolism and decrease TG secretion from liver by decreasing β-oxidation of fatty acids leading to accumulation in the liver [11, 12]. The high fat diet prepared incorporating the cholesterol and cholic acid increase the level of total cholesterol, triglycerides, atherogenic index and decrease total HDL-C in Wistar albino adult male rats [13, 15] and increase the levels of serum enzymes like LDH, AST, ALT, ALP, creatinine, albumin [16, 18]. Similar results were obtained in the present study by inducing the hyperlipidemia in rats. The administration of a high-cholesterol diet is known to cause steatohepatitis in experimental animals, an injury which is attributed to damage of the hepatocyte membrane and increase the levels of liver enzymes [16, 19].

In the treatment groups significant decrease ($P \leq 0.05$) in the mean cholesterol values were observed in all the treatment groups compared to diet control group but the values were still significantly higher ($P \leq 0.05$) than normal control on 42nd day of the treatment. On 56th day of the treatment the mean values in the atorvastatin and aged garlic with atorvastatin treatment groups were reduced to normal levels.

Triglyceride values were significantly ($P \leq 0.05$) decreased in AGE and mixed treatment groups. High density lipoproteins improved significantly in all the treatment groups compared to diet control. In all the treatment groups a significant ($P \leq 0.05$) improvement (reduction) in the low density lipoprotein values was seen compared to high fat diet control on both day 42 and 56 of the study and the reduction was more in atorvastatin and mixed treatment groups than aged garlic treatment group. Very low density lipoproteins showed significant improvement in aged garlic and mixed treatment groups but atorvastatin treatment did not reduce VLDL levels.

Garlic alters the portions of lipoprotein cholesterol fractions, which is attributed to excretion of cholesterol and cholesterol metabolites and change in the endogenous synthesis of cholesterol by the garlic and inhibition of hepatic fatty acid synthesis [20]. The water soluble organosulfur compounds in the Kyolic inhibit cholesterol synthesis with their concerted effects [21].

Aged garlic extract and SAC decreases the serum triglycerides by mediating the release of endothelium bound lipoprotein lipase, which hydrolyses TG to into fatty acids and the elevated levels of HDL is attributed to increased activity of Lecithin- cholesterol acyl transferase, which plays a key role in incorporating free cholesterol in to HAD and transferring back in to VLDL and IDL which is taken back by liver cells [22]. These compounds lower hepatic cholesterol synthesis by inhibiting acetate incorporation into cholesterol, decreasing the activities of 3-hydroxy -3-methyl-glutaryl-CoA reductase in the liver and inhibit the [2,3H] glycerol incorporation into TG [23, 24].

Table 1: Mean (± SE) values of Different serum lipidps (mg/dL) in various groups on 42nd and 56th of Experiment

Parameter	Days post	Group- I	Group- II	Group-III	Group- IV	Group –V	Group –VI
CHO	Day 42	109.33±2.77^{ab}	174.00±5.53^{e}	98±1.65^{a}	141.33±3.37^{d}	124.16±2.20^{c}	122.16±1.406b^{c}
	Day 56	114.83±4.29^{a}	191.16±5.75^{c}	107.00±4.13^{a}	133.66±2.31^{b}	118.33±2.81^{ab}	118.50±1.83^{ab}
TGS	Day 42	89.00±3.65^{d}	179.00±9.98^{a}	61.50±2.12^{c}	129.00±4.70^{b}	161.83±5.49^{a}	129.50±1.83^{b}
	Day 56	93.16±6.31^{ac}	197.16±12.81^{b}	67.50±3.17^{a}	124.50±8.00^{d}	167.00±5.31^{b}	123.33±2.40^{cd}
HDL	Day 42	49.16±2.54^{b}	34.33±1.64^{a}	74.33±3.55^{c}	39.50±1.08^{b}	44.33±1.82^{b}	49.16±3.34^{b}
	Day 56	47.50±2.02^{a}	29.00±1.16^{b}	67.33±2.44^{c}	46.16±1.42^{a}	49.83±1.83^{a}	52.10±1.96^{a}
LDL	Day 42	42.37±4.05^{a}	103.87±5.27^{d}	11.37±3.61^{c}	76.03±3.60^{b}	47.47±3.93^{a}	47.10±2.64^{a}
	Day 56	48.70±3.06^{ab}	122.73±6.74^{d}	26.17±5.10^{c}	62.60±3.21^{a}	35.10±3.15^{bc}	41.83±2.81^{bc}
VLDL	Day 42	17.80±0.73^{c}	35.80±1.99^{a}	12.30±0.45^{d}	25.80±0.94^{b}	32.37±1.09^{a}	25.90±0.36^{b}
	Day 56	18.63±1.26^{ac}	39.43±2.56^{b}	13.50±0.63^{e}	24.90±1.60^{d}	33.40±1.06^{b}	25.90±0.40cd

The ALT values in AGE and mixed treatment groups improved significantly but in the atorvastatin treatment group the values were significantly higher compared to normal control. A mild but not significant improvement in the mean AST values was seen in all the treatment groups compared to diet control group. GGT, creatinine and BUN values did not show much variation among the control and treatment groups. The decreased ALT in AGE treatment groups could be due to protection by AGE against damaging effects of hyperlipidemia induced by the high fat diet there by improving the hepatic function. In the present study, gamma glutamyl transferase (GGT), creatinine and BUN levels did not vary indicating normal bile duct epithelial and kidney function. The improvement with respect to AST was attributed to reduced lipid peroxidation and improved antioxidant profile and resulting membrane integrity of the cells by the organosulphar compounds present in the garlic values may be due to the improved mitochondrial membrane integrity [25].

Table 2: Mean (± SE) values of ALT,AST,GGT, Creatinine and BUN in various groups on 42nd and 56th day of experiment

ALT (IU/L)	Day 42	47.83±2.51^{ac}	79.48±2.80^{b}	43.61±2.77^{a}	52.16±1.80^{ac}	69.66±1.24^{b}	54.83±2.79^{c}
	Day 56	43.68±3.52^{ab}	95.03±4.14^{e}	38.78±2.24^{a}	53.78±1.21^{bc}	73.41±1.12^{d}	61.08±2.58^{c}
AST (IU/L)	Day 42	122.53±4.35^{ab}	146.33±4.64^{c}	106.51±4.74^{a}	127.93±3.05^{bc}	139.50±3.80^{bc}	128.78±5.69^{bc}
	Day 56	120.70±4.42^{ab}	143.35±7.69^{c}	112.15±3.55^{a}	131.26±2.59ab^{c}	136.50±4.32^{bc}	132.45±3.94^{bc}
GGT (IU/L)	Day 42	3.36±0.32	3.58±0.57	4.43±0.38	3.68±0.64	3.58±0.16	3.41±0.47
	Day 56	4.30±0.60	4.01±0.22	3.85±0.33	3.70±0.62	3.56±0.16	3.16±0.47
CRT(mg/dL)	Day 42	1.53±0.02	1.58±0.05	1.40±0.06	1.56±0.07	1.52±0.05	1.49±0.06
	Day 56	1.49±0.03	1.51±0.07	1.55±0.02	1.50±0.06	1.48±0.05	1.41±0.06
BUN(mg/dL)	Day 42	68.90±3.00^{ab}	72.35±3.97^{ab}	75.85±1.64^{a}	68.88±2.08^{ab}	61.10±1.51^{b}	64.26±4.21^{ab}
	Day 56	64.66±3.81	58.15±3.53	60.40±2.19	69.45±2.41	62.88±1.51	61.93±4.21

The combined treatment of atorvastatin and garlic induces more pronounced hypolipidemic effect than single treatment which is attributed to their synergistic effect via similar mechanisms of action. Inhibition of the HMG-CoA reductase, the main mechanism of action of atorvastatin may be augmented by organosulphar compounds of garlic as reported by [25] and inhibition of activity on $CYP3A_4$ and P-*gp*, which increase the oral availability of atorvastatin due to inhibition of first-pass metabolism [26].

Conclusion

This study highlighted the beneficial effects of aged garlic extract at 200mg/kg bw and mixed treatment at half the dose (100mg/kg) with atorvastatin (7.5mg/kg) in improving the serum lipid profile and enzymes in induced hyperlipidemia in male Wistar rats and possible mechanisms underlying in the improvement.

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