Certain biochemical effects of garlic oil on rats maintained on high fat-high cholesterol diet

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Summary. The feeding of a high fat-high cholesterol (HF-HC) diet to normal rats for 1 month increased the lipid components cholesterol and triglyceride in serum, liver and kidneys and decreased the serum albumin very significantly. Administration of garlic oil (100 mg/kg b. wt/day) for 1 month together with the HF-HC diet to another group almost nullified the lipid-increasing and albumin-decreasing effects of that diet. The reduction in total lipids, cholesterol and triglycerides and the restoration to normal level of serum albumin were highly significant in the garlic oil group. Adipose tissue triglyceride lipase activity was significantly increased in both the above groups with a much greater rise in the oil group.

Hyperlipidemia is a high risk factor and it may lead to ischemic heart disease[1]. There is a widespread search for a drug which may control both hypercholesterolemia and hypertriglyceridemia. The hypocholesterolemic effect of garlic (*Allium sativum* Linn) was reported by Tempel[2]. Later on, one of the authors[3] reported the hypolipidemic effect of garlic. During the last decade the hypolipidemic effect of garlic has been confirmed by many workers[4-8]. The beneficial effects of garlic extracts or garlic oil[4,9-11] in controlling hyperlipidemia and hyperglycemia urged Chang and Johnson[12] to study the effects of garlic in animals. They reported that the addition of garlic to a sucrose + cholesterol diet in rats effectively prevented the rise in serum and liver cholesterol, triglycerides and free fatty acids. Moreover, garlic addition decreased blood sugar with a concomitant increase in liver glycogen. Tempel[2] and one of the authors[13] ascribed the hypolipidemic action of garlic to its sulphur compounds. The present work describes certain biochemical effects of garlic oil, which is composed of diallyl disulphide[14], on rats maintained on a high fat-high cholesterol diet.

Material and methods. Garlic oil was extracted from raw cloves of garlic which was sliced, crushed and soaked in diethyl ether for 48 h, after which the supernatant was decanted. Extraction of the oil from the garlic residue left behind was repeated thrice with more solvents. The oil was recovered from the combined extracts by distilling off the solvent at 40 °C. The oil was shaken with 20 vol. of redistilled petroleum ether (boiling point 40-60 °C) and centrifuged. The clear supernatant was removed and the petroleum ether soluble fraction of the oil was prepared by distilling off the solvent at 60 °C. The yield was 500 mg/kg wet wt. Gaschromatographic analysis showed that this oil is identical with diallyl disulphide, as reported before[14].

A standard diet as well as a high fat-high cholesterol (HF-HC) diet were prepared by mixing appropriate amounts of wheat flour, milk powder, margarine and millet husk with salt mixture[15] and vitamins (A, B_1, B_2, B_6, C, and D_2). Standard diet consisted of 73% carbohydrate, 16% protein, 3% satured fat, 5% fiber, 2% salt mixture and 1% vitamin tablets. The high fat-high cholesterol diet consisted of 56% carbohydrate, 16% protein, 18% saturated fat, 2% cholesterol, 5% fiber, 2% salt mixture and 1% vitamin tablets. Male albino Wistar rats with an average body weight of 100 g were divided into 3 groups of 8 rats. One group, fed ad libitum on the standard diet was kept as normal. The 2nd group was maintained on the HF-HC diet, while the 3rd group was maintained on the HF-HC diet + garlic oil (100 mg/kg b. wt/day). The diet was fed ad libitum and the oil was administered as a saline suspension through a stomach tube. After feeding the oil on the 30th day, the rats in all the groups were weighed again and they were kept for overnight fasting. Next day the rats were sacrificed by decapitation. Blood, liver, kidney and epididymal fat pads were separated immediately for various estimations. Blood sugar was estimated by the method of Asatoor and King[16]. In serum, liver and kidney, total cholesterol, triglycerides and total proteins and, in addition, in serum, albumin and in liver, total lipids were estimated as described below. Lipid extracts obtained from liver and kidneys by the method of Entenman[17] and whole serum were used for estimation of cholesterol by the method of Henly[18]. For the estimation of triglyceride in terms of glycerol[19] lipid extracts of tissues and serum were prepared and saponified and the triglyceride glycerol was estimated by the method of Burton[20]. Liver total lipids were gravimetrically estimated from a sample of liver lipid extract[17] as described earlier[13]. Serum albumin and total proteins were determined by the method of Reinhold[21]. Liver and kidney homogenate proteins were determined by the method of Lowry et al.[22]. Triglyceride lipase activity of the epididymal adipose tissue was determined according to the method of Rudman et al.[23]. Glycerol release from the adipose tissue into the incubation medium was used as the index of triglyceride lipase activity[24].

Results. The results are given in the table. The HF-HC diet increased the lipid components in serum, liver and kidneys of the rats very significantly. The cholesterol levels in serum and kidneys increased by 100% and in the liver by 5 times the normal.

The increase in total lipids in the liver was 100% ($p < 0.001$). The serum and liver triglyceride levels increased by 1.8 times and kidney triglyceride levels by 2.4 times the normal ($p < 0.001$). However administration of garlic oil along with the HF-HC diet almost nullified the lipid-increasing effect of the latter. Total cholesterol in serum, liver and kidneys of the oil-fed group was reduced to almost normal levels. The triglycerides in the same group were reduced to values below normal. The reductions in cholesterol and triglyceride values were highly significant ($p < 0.001$). Total lipids in the liver also decreased to normal in the oil-fed group ($p < 0.002$). HF-HC diet reduced serum albumin by 26% ($p < 0.001$). The total proteins, however, did not show any significant decrease. Serum albumin was restored almost to the normal level when garlic oil was given with the HF-HC diet and the effect was significant ($p < 0.001$). However, the total protein did not change. Liver and kidney proteins were not decreased by the HF-HC diet or by the HF-HC diet + garlic oil.

HF-HC diet did not change the fasting blood sugar values of the rats. However, there was a non-significant decrease in the fasting blood sugar level of the garlic oil group. HF-HC diet increased the adipose tissue triglyceride lipase activity of rats very significantly ($p < 0.01$). Administration of garlic oil along with HF-HC diet further increased this enzyme activity and it was significant ($p < 0.01$). With reference to increase in body weight there was no significant difference between the groups.

Effects of garlic oil on high fat-high cholesterol fed rats. Values are mean ± SD of 8 rats

Parameters studied	Normal rats	Rats fed high fat-high cholesterol diet	Rats fed high fat-high cholesterol + garlic oil diet	Percentage of $\frac{\text{HF-HC + oil}}{\text{HF-HC}}$ values
Blood sugar (mg/100 ml)	73.0 ±10	70.0 ±12	60.0 ±16	85
Serum				
Total cholesterol (mg/100 ml)	69.0 ±9	135.0 ±15[b]	76.0 ± 8[c]	56.3
Triglyceride glycerol (mg/100 ml)	25.0 ±3	44.0 ± 4[b]	16.0 ± 3[c]	36.4
Albumin (g/100 ml)	5.7 ± 0.12	4.2 ± 0.18[b]	5.2 ± 0.11[c]	124.0
Total protein (g/100 ml)	7.8 ± 0.4	7.4 ± 0.16	7.6 ± 0.18	102.0
Liver				
Total cholesterol (mg/100 g)	138.0 ±15	653.0 ±20[b]	125.0 ±12[c]	19.1
Triglyceride glycerol (mg/100 g)	120.0 ±10	216.0 ±25[b]	60.0 ± 8.0[c]	27.7
Total lipid (g/100 g)	3.12 ± 0.6	6.4 ± 1.1[b]	3.05 ± 0.8[d]	48.4
Total protein (g/100 g)	14.5 ± 0.3	15.5 ± 0.45	15.0 ± 0.4	96.1
Kidney				
Total cholesterol (mg/100 g)	103.0 ±4	214.0 ± 7[b]	86.0 ± 5.0[c]	40.2
Triglyceride glycerol (mg/100 g)	35.0 ± 5.8	84.0 ±10[b]	19.0 ± 2.0[c]	22.6
Total protein (g/100 g)	9.1 ± 0.2	8.9 ± 0.3	9.0 ± 0.2	101.1
Triglyceride lipase activity of epididymal fat pad (mg glycerol/g/h)	*0.095 ± 0.02*	*0.159 ± 0.01*[a]	*0.178 ± 0.01*[c]	112.0

Level of significance between the groups was calculated by Student's t-test; significant values in HF-HC group as compared to normal group are: [a]$p < 0.01$; [b]$p < 0.001$; and significant values in HF-FC + garlic oil group as compared to HF-HC group are: [c]$p < 0.001$; [d]$p < 0.002$.

Discussion. The rise in cholesterol in serum, liver and kidneys may be due to increased uptake of exogenous cholesterol and subsequent deposition as a result of HF-HC feeding and decreased cholesterol catabolism as evidenced by a reduction in bile acid production and turnover after high fat feeding[25,26]. Both in the serum and in the tissues garlic oil prevented an increase of cholesterol. The rise in triglycerides in serum and tissues of rats fed the HF-HC diet may be due to endogenous synthesis[27] and decreased hepatic oxidation[28]. Our results on the effects of garlic oil are in agreement with the findings of Chang and Johnson[12]. The mechanism by which garlic oil prevented the rise in cholesterol, triglycerides and total lipids in HF-HC fed rats is not yet clear. However there is strong evidence to suggest the following mechanisms, based on the reactions of disulphides like the diallyl disulphide of garlic oil[29].

The organic disulphide compounds can undergo exchange reactions with thiol compounds[30,31].

$$C_3H_5{-}S{-}S{-}C_3H_5 + R{-}SH \rightarrow C_3H_5{-}S{-}S{-}R + C_3H_5SH$$

Where R–SH stands for all thiol group proteins, enzymes etc. Thus the availability of SH group enzymes, such as CoASH, the multienzyme complex of fatty acid synthesis, and of HMGCoA reductase, the rate-limiting enzyme for cholesterol synthesis, may be reduced by the action of the garlic oil substance diallyl disulphide[14,29]. This could bring about a reduction in the lipid components of tissues and serum.

A second possible mechanism of action of the garlic oil is to oxidize NADPH, which is necessary for lipid synthesis. Diallyl disulphide is an unsaturated compound which reacts as follows[32].

$$CH_2{=}CH{-}CH_2{-}S{-}S{-}CH_2{-}CH{=}CH_2 + 3\ NADPH + 3\ H^+ \rightarrow 2\ C_3H_7SH + 3\ NADP^+$$

Therefore the availability of reducing equivalents for fatty acid synthesis may be decreased by garlic oil, reducing the rate of fatty acid synthesis. However, further proof of the mechanism of action of the oil is needed.

How the HF-HC diet could reduce serum albumin in rats is not clear. Total serum proteins, however, did not fall significantly, probably because of the rise in one or more of the globulin fractions[33]. How garlic oil restored the serum albumin level to normal in HF-HC fed rats is again another puzzle. The total serum proteins did not change in this group either.

The increase in adipose tissue triglyceride lipase activity in HF-HC fed rats was in conformity with the findings of others[34] who used sucrose as the lipogenic diet in their experiments. Administration of garlic oil along with the HF-HC diet further increased the triglyceride lipase activity by some unknown mechanism.

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Bombykol biosynthesis from deuterium-labeled (*Z*)-11-hexadecenoic acid

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Summary. (*Z*)-[11,12d_2]-11-Hexadecenoic acid was applied topically to the pheromone gland of silkworm pupa. After eclosion, the gland components were analyzed by capillary GC-MS. (*Z*)-11-Hexadecenoic acid, a characteristic fatty acid of the gland, was found to be a precursor of bombykol, (*Z*)-11-hexadecenol and bombyk acid.

Biosynthesis of bombykol, the sex pheromone of the female silkworm moth, was studied by Inoue and Hamamura 10 years ago using radiolabeled (1-^{14}C) palmitic acid. They demonstrated that palmitic acid might be a precursor of bombykol[2].

Previously we reported the presence of a characteristic fatty acid, (*Z*)-11-hexadecenoic acid, [(*Z*)-11-HDA], in the pheromone gland of female silkworm pupae and moths and proposed a possible biosynthetic route for bombykol, from palmitic acid via (*Z*)-11-HDA[3].

We report here some experiments using deuterium-labeling and capillary GC and GC-MS which verify that (*Z*)-11-HDA is a precursor of (*Z*)-11-hexadecenol[4], bombyk acid, bombykol, and its (*E,E*) isomer[5].

Materials and methods. The female pupae were kept at 25 °C and 2 days before eclosion, the oviposition and pheromone gland were everted by pressing the abdomen with the fingers. d_2-(*Z*)-11-HDA (0.3 µl) was applied topically to the gland. The treated female pupae were kept at 25 °C. 8 h after eclosion, the pheromone glands of adult female moths were excised and extracted with ether. A half aliquot of the extract was subjected to capillary GC and GC-MS analysis (JEOL D-300 with HP-5740 GC attached; PEG 20 M g-SCOT 25 m × 0.28 mm i.d.) to identify aliphatic alcohols. To another half aliquot including the glands, chloroform-methanol (2:1) was added, and the extract was subjected to acid methanolysis to generate methyl esters from acylglycerides. The resulting methyl esters were analyzed by the same methods as those used for the alcohols.

11d,12d (*Z*)-11-HDA was synthesized by coupling 1-hexyne with decamethylenbromohydrin. The produced hexadecynol was treated with deuterium gas in catalytic partial reduction. The resulting d_2-hexadecenol was oxdized to the d_2-HDA.

Results and discussion. Fatty acids. Fatty acids of pheromone gland treated with 11*d*, 12*d* (*Z*)-11-HDA were analyzed by capillary GC-MS. 6 major and 1 minor peaks were observed on the total ion chromatogram and identified by gas chromatographic retention times and by mass spectrometry. Ions with m/z 268(M^+), 237(M-31), 236(M-32), and 194(M-74) which are the 'diagnostic ions of methyl hexadecenoate' and those with m/z 270(M^+), 239, 238, and 196 which are the 'diagnostic ions of d_2-methyl hexadecenoate' were observed on the same mass spectrum of the peak at tR. 10.0 min. Molecular ion of methyl bombykate was m/z 266, but ion m/z 268 which is on '(M^+) of d_2-methyl bombykate' was also present in the same mass spectrum of the minor peak at tR. 11.9 min. The change of m/z 266 to 268 on the spectrum was about 15%. Ion m/z 268 was not observed in the mass spectrum of methyl bombykate that originated from the pheromone glands of the untreated female. These data suggest that the bombyk acid is synthesized from (*Z*)-11-HDA in the pheromone gland.

Alcohols. The pheromone gland components of adult female moths were analyzed under the same conditions as those for fatty acid analysis. Four diagnostic peaks (peak A, tR. 9.0 min; peak B, tR. 9.5 min; peak C, tR. 11.6 min; peak D, tR. 12.1 min) were observed on the total ion chromatogram. On the mass spectrum of peak B, ion m/z 222 and 224 were observed. Ion m/z 222 was a (M-18) ion of (*Z*)-11-hexadecenol which was one of the usual components in the pheromone gland. The relationship between the retention times of peaks B and A (hexadecanol) on capillary GC indicated that peak B was (*Z*)-11-hexadece-