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Regression of preestablished cholesterol gallstones by dietary garlic and onion in experimental mice

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Abstract

We have recently reported the health beneficial potential of dietary garlic and onion in reducing the incidence and severity of cholesterol gallstone (CGS) during its experimental induction in mice. In the current study, the efficacy of dietary garlic and onion in regressing preestablished CGS was investigated in experimental mice. After inducing CGS in mice with a lithogenic diet for 10 weeks, they were maintained on basal diets containing 0.6% dehydrated garlic or 2% dehydrated onion for a further 10 weeks. Dietary garlic and onion, either raw or heat processed, regressed preformed CGS in mice up to 53% to 59%, whereas the regression in the basal control diet group was only 10%. The antilithogenic potency of garlic was decreased by its heat processing, but not in the case of onion. Biliary cholesterol was significantly decreased in garlic- and onion-fed animals. Biliary cholesterol saturation index and hydrophobicity index were significantly lowered by dietary garlic and onion. Serum and liver cholesterol levels were decreased by feeding these spices during post-CGS induction period. Hepatic hydroxymethylglutaryl–coenzyme A reductase activity was increased after feeding garlic and onion, whereas activities of the cholesterol-degrading enzymes cholesterol-7α-hydroxylase and sterol-27-hydroxylase were increased in spice-fed groups. These results indicate that feeding garlic and onion effectively accelerates the regression of preformed CGS by promoting cholesterol desaturation in bile. This observation is significant in the context of evolving dietary intervention strategy to address regression of existing CGS and stopping the possible recurrence.

1. Introduction

The main constituents of bile, which plays an important role in the digestion and absorption of dietary lipids, are bile acids, phospholipids, cholesterol, and small amounts of protein. Cholesterol gallstone (CGS) pathogenesis is a primary disorder arising out of altered hepatic and biliary cholesterol homeostasis. Gallstones are formed in the gallbladder because of precipitation of cholesterol, bilirubin, and calcium salts in bile. Majority of gallstones are contributed by cholesterol, and very small numbers of gallstones are primarily composed of calcium salts of bilirubin and phosphate.

Pathologic conditions that generally precede the occurrence of CGS are lithogenic bile, gallbladder stasis, and short nucleation time. Lithogenicity of bile is determined by relative concentration of 3 main components, namely, bile acids, phospholipids, and cholesterol. Generally, lithogenic bile occurs with disruption of cholesterol homeostasis, leading to increased cholesterol secretion and subsequent supersaturation of cholesterol in bile [1,2]. Gallbladder stasis increases the opportunity for concentration of supersaturated bile in the gallbladder to form gallstones. It is universally accepted that cholesterol supersaturation in bile is the prerequisite step in the onset of CGSs. The onset of CGS is associated with various physicochemical disturbances in bile, which include excess secretion of cholesterol and reduced secretion of bile acids by the liver into bile and increased absorption of water from the gallbladder, thus creating a hydrophobic environment. These series of events lead to supersaturation of bile with cholesterol, a prerequisite for CGS formation [3].

Several studies involving animal models to evaluate the role of dietary components in preventing CGS have been reported. It has been shown that dietary proteins, carbohydrates, fiber, and fat play a role in the induction of CGSs

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[4-6]. On the other hand, role of dietary factors in the regression or amelioration of already existing CGS is not studied extensively. Spices—garlic, onion, fenugreek, red pepper, and turmeric—have been documented to be effective as hypocholesterolemic agents under conditions of experimentally induced hypercholesterolemia and hyperlipidemia [7]. Earlier, it has been shown that curcumin of turmeric and capsaicin of red pepper when included in the diet reduced the induction of CGSs in mice [8]. Dietary fenugreek seeds as well as the above 2 spice bioactive compounds have been evidenced to regress preformed CGSs in experimental mice when included in the diet after CGS induction [9,10]. Dietary garlic and onion have been shown to be hypocholesterolemic and also act as cholagogue [11-13]. We have recently evidenced the antilithogenic influence of dietary garlic and onion in experimental mice in terms of reducing the incidence of atherogenic diet–induced CGS [14]. However, there is no information on the possible role of garlic and onion in the regression of preformed CGSs.

Generally, CGS treatment involves lithotripsy, cholecystectomy, or the use of drugs such as ursodeoxycholate. But these treatments have limitations, and the chances of reoccurrence of stones are higher [15]. Hence, it is desirable to search for alternative noninvasive treatments such as dietary intervention to regress the existing CGS. In this context, the present study has evaluated the role of 2 hypocholesterolemic *Allium* spices—garlic and onion—in regressing the existing CGS in experimental mice. These test spices were evaluated in both raw and heat-processed forms.

2. Materials and methods

2.1. Materials

Cholesterol, dipalmitoyl phosphatidylcholine, bile salts, boron trifluoride in methanol, bovine serum albumin, ethylenediaminetetraacetic acid, hydroxymethylglutaryl–coenzyme A (HMG-CoA), dithiothreitol, triethanolamine, nicotinamide adenine dinucleotide phosphate reduced (NADPH), 3α-hydroxysteroid dehydrogenase, nicotinamide adenine dinucleotide (NAD), standard bile acids kit (conjugated and unconjugated), Tris, triolein, and α-cellulose were purchased from Sigma Chemical (St Louis, MO). Heparin and $MnCl_2$ were obtained from SISCO Research Laboratory (Mumbai, India). Casein was purchased from Nimesh (Mumbai, India). All solvents used were of analytical grade and were distilled before use. Garlic and onion freeze-dried powders were a generous gift from Indo Nissin Food, Bangalore, India.

2.2. Animal diets

The animals were fed with AIN-76 semipurified diet. The basal control diet consisted of sucrose, 65%; casein, 20%; cellulose, 5%; AIN-76 mineral mix, 3.5%; AIN-76 vitamin mix, 1%; DL-methionine, 0.3%; choline chloride, 0.2%; and refined peanut oil, 5%. Lithogenic (LG) diet was prepared by supplementing 0.5% cholesterol and 0.25% bile salts (1:1 mixture of sodium cholate and sodium deoxycholate) to the AIN-76 basal diet. Various test diets were prepared by adding garlic powder (0.6%) or onion powder (2%) to the AIN-76 basal diet. The diets were made isocaloric by varying the sucrose content. Heat processing of garlic and onion was done by adding the respective freeze-dried powder to the boiling water and boiling for 15 minutes with constant stirring as practiced normally in the Indian culinary, and these were used as heat-processed spices in these experiments. All these diets were prepared by mixing the ingredients in a mechanical mixer, and pellets were prepared using hand-operated pelletizer. Diets were stored at 4°C in air-tight containers.

2.3. Animal treatment

Animal experiments were carried out taking appropriate measures to minimize pain or discomfort and with due approval from the Institutional Animal Ethics Committee. Four-week-old male albino mice (OUTB/Swiss Albino/IND/CFT [2c]) from Experimental Animal Production Facility of this institute, weighing 22 ± 2 g, were grouped and housed in polypropylene cages with saw dust as bedding. Groups of animals were fed ad libitum with LG diet and various test diets and had free access to water throughout the experimental period. Body weights were recorded at the beginning of the experiment and at the end of every week.

Groups of male mice (n = 226) were initially fed with LG diet for 10 weeks (Fig. 1). At the end of 10 weeks, 10 animals were randomly selected and killed to confirm the formation of CGS. Remaining animals were divided into 2 groups, and each group was further subdivided into 6 subgroups. Among them, 1 subgroup was continued on LG diet, another subgroup was fed with AIN-76 basal diet, and the other 4 subgroups were fed with basal diet containing *Allium* spices as given in Fig. 1. The first group of animals (6 subgroups) was fed with these experimental diets for 5 weeks, whereas the second group of animals (6 subgroups) was fed for 10 weeks. Normal control animals (n = 36) were fed only AIN-76 basal diet throughout the duration of experiment (ie, during the initial 10 weeks and the subsequent 5 and 10 weeks).

At the end of the feeding period, animals were fasted overnight and killed under ether anesthesia. Blood was drawn immediately by cardiac puncture, and the serum was separated by centrifugation for further analysis. Other organs were quickly excised, washed with ice-cold saline, blotted dry, weighed, and stored at −20°C until further analysis. Cholecystectomy was performed, and gallbladders were carefully collected. The volume of bile was noted, and the weight of the gallbladder along with stones was recorded. The gallbladders were evaluated for CGS under magnifying lens for the presence of gallstones by 4 individuals unaware of dietary treatments. The grading of stones was done on a 5-point scale [16]. The bile from the gallbladders was pooled and stored at −20°C until further analysis.

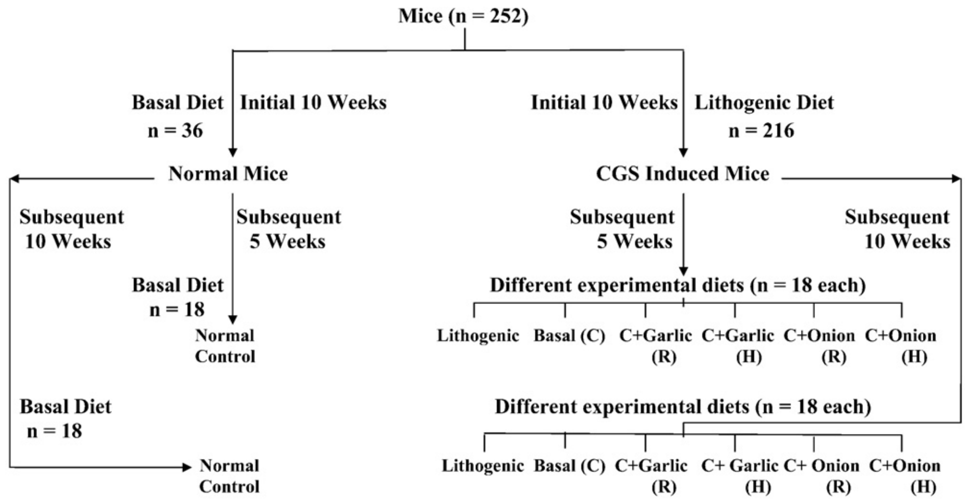

Fig. 1. Experimental design for studying the regression of CGS in mice by dietary garlic and onion. Lithogenic diet: 0.5% cholesterol; garlic: 0.6% freeze-dried powder; onion: 2% freeze-dried powder. R indicates raw; H, heat processed.

2.4. Analysis of lipids

Gallstones were crushed to powder along with the fluid bile. Such a suspension of crushed stones in the bile was used for lipid extraction. Biliary lipids were extracted by the method of Bligh and Dyer [17], and the chloroform phase was used for lipid analysis. The upper methanolic phase was used for the estimation of total bile acids using 3α-hydroxysteroid dehydrogenase [18]. Serum and liver lipids were extracted by the method of Folch et al [19]. Cholesterol levels were quantitated by the method of Searcy and Bergquist [20]. Phospholipids were measured by the method of Stewart [21] using dipalmitoyl phosphatidylcholine as reference standard. Triacylglycerol was estimated by the method of Fletcher [22]. Conjugated and unconjugated bile acids were quantified according to Rossi et al [23]. The average hydrophobic and hydrophilic balance of biliary bile salts was quantified by means of bile salt monomeric hydrophobicity index (HI) [24]. The cholesterol saturation index (CSI) of bile was calculated according to Carey [25].

Phospholipid-associated fatty acids were analyzed as methyl esters prepared using boron trifluoride in methanol as described by Morrison and Smith [26] and analyzed by gas chromatography (Shimadzu 14B, fitted with a flame ionization detector; Kyoto, Japan) using fused silica capillary column 25 m × 0.25 mm (Parma bond FFAP-DF-0.25; Machery-Nagel GmbH Co., Duren, Germany). The operating conditions were as follows: initial column temperature, 160°C; injector temperature, 210°C; and detector temperature, 250°C. Column temperature was programmed to rise at 6°C per minute to the final temperature of 240°C. Nitrogen gas was used as the carrier. Individual fatty acids were identified by comparing with retention times of standards (NU-Chek Prep, Elysian, MN) and were quantitated by online Choromatopack CR-6A integrator (Shimadzu Corp. Inc., Kyoto, Japan).

2.5. Activities of enzymes of cholesterol metabolism

Hydroxymethylglutaryl–CoA reductase activity in liver was assayed by following the formation of CoA [27]. Activities of cholesterol-7α-hydroxylase and sterol-27-hydroxylase in liver were assayed as described by Petrack and Latario [28]. Biliary proteins and protein in liver were analyzed according to Lowry et al [29].

2.6. Statistical analysis

Statistical analysis was carried out using GraphPad (La Jolla, CA) Prism statistical software. Results are analyzed by 1-way analysis of variance, and the significance level was calculated using Tukey-Kramer multiple comparison test. Results are considered as significant at $P < .01$.

3. Results

3.1. Effect of feeding garlic and onion for 5 and 10 weeks on the regression of preformed CGS in mice

Feeding of LG diet for 10 weeks had successfully induced CGS in mice, as confirmed by physically examining the gallbladders of 10 animals randomly picked among the lot. The various experimental spice diets were started at this point in time for a further period of either 5 or 10 weeks to evaluate the beneficial antilithogenic influence with regard to regression of preformed CGS. The effect of feeding garlic and onion for 5 and 10 weeks on CGS score in animals with previously induced CGS is presented in Fig. 2. The CGS score that was 3.80 in LG group and 3.65 in basal control group was reduced to 2.08 and 3.25 in garlic-fed animals (raw and heat processed) and to 2.70 and 2.0 in onion-fed animals (raw and heat processed) at 5 weeks. The same was even lower at 10 weeks (1.85 and 2.56 in the case of garlic, 2.3 and 1.63 in the case of onion).

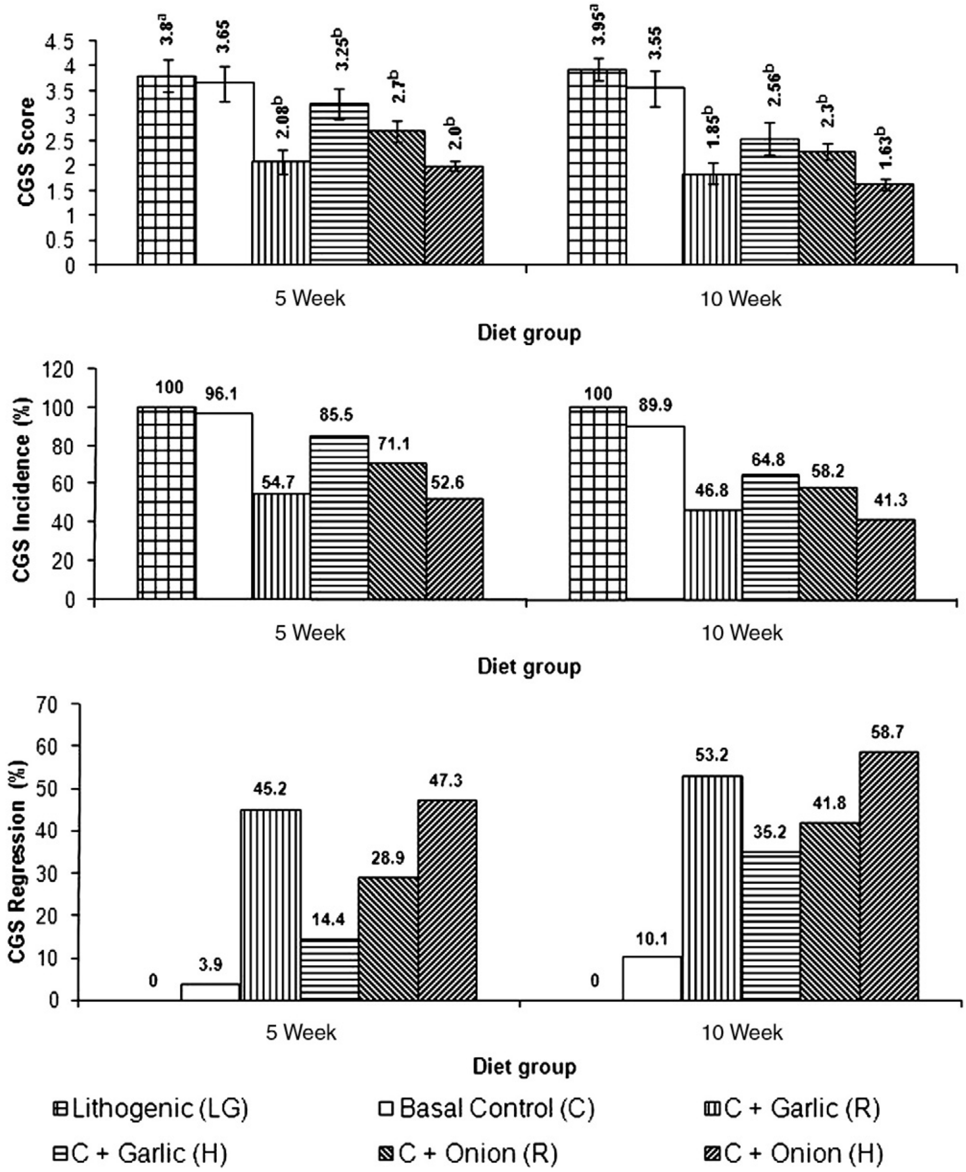

Fig. 2. Effect of feeding garlic and onion for 5 and 10 weeks on CGS score in CGS-prevailing mice. The CGS score values are mean ± SD of 18 mice per group. Animals in all diet groups except normal control were fed initially with LG diet for 10 weeks to induce CGS. The CGS score in LG group was statistically different ($P < .01$) when compared with normal control group that was maintained on basal control diet through out (CGS score = 0). The CGS scores in garlic- and onion-fed groups were statistically different when compared with basal control (C) group ($P < .01$).

Thus, regression of CGS brought about by dietary garlic was 45% and 14%, whereas onion produced a regression of 29% and 47% after 5 weeks. Regression of CGS brought about by dietary garlic was 53% and 35%, whereas onion produced 42% and 59% regression after 10 weeks, as compared with a mere 4% and 10% in animals allowed to consume basal control diet. It is significant to note that control diet feeding after CGS formation did not alter any of the parameters studied.

The body weights of the animals were comparable among various groups except for a slight increase in LG group (Table 1). Liver weight was increased in LG group by 2.2-fold compared with normal control group. Garlic and onion feeding during post-CGS induction significantly lowered the liver weights as compared with the animals maintained on basal control diet.

3.2. Effect of feeding garlic and onion for 5 and 10 weeks on serum lipid profile in CGS-prevailing mice

Influence of feeding garlic and onion for 5 and 10 weeks during post-CGS induction period on serum lipid profile is presented in Table 2. Serum cholesterol content in the mice fed LG diet during the post-CGS induction period remained higher compared with uninduced control group at both the time intervals. There was no significant reduction in serum cholesterol during discontinuation of LG diet during post-CGS induction period (animals fed

Table 1
Effect of feeding garlic and onion for 5 and 10 weeks on body weight and liver weight

Diet group	Body weight (g)		Liver (g)	
	5 wk	10 wk	5 wk	10 wk
Normal control[a]	43.0 ± 2.23	44.5 ± 2.35	1.43 ± 0.12	1.32 ± 0.15
LG	48.7 ± 3.33	46.3 ± 2.26	3.10 ± 0.21*	3.52 ± 0.31*
C	45.6 ± 2.36	44.6 ± 2.36	2.84 ± 0.31	2.64 ± 0.31†
C + garlic (R)	43.0 ± 2.07	41.6 ± 3.10†	2.43 ± 0.28†	2.11 ± 0.18†
C + garlic (H)	43.7 ± 3.56	42.9 ± 2.18	2.24 ± 0.58†	2.09 ± 0.18†
C + onion (R)	42.1 ± 2.17	40.2 ± 1.93†	2.40 ± 0.47†	2.15 ± 0.22†
C + onion (H)	43.2 ± 1.52	40.3 ± 1.36†	2.04 ± 0.37†	1.92 ± 0.20†

Values are mean ± SD of 18 mice per group. Animals in all diet groups except normal control were fed initially with LG diet for 10 weeks to induce CGS. C indicates basal control; R, raw; H, heat processed.

[a] Animals in normal control group were maintained on basal control diet throughout.

* Statistically significant when compared with normal control group at $P < .01$.

† Statistically significant when compared with basal control group at $P < .01$.

basal control diet). On the other hand, treatment with diets containing garlic or onion during post-CGS induction period produced decreases of the order of 22% (raw garlic), 10%, and 22% (raw and heat-processed onion, respectively) when compared with basal control group. Serum triglyceride was higher in the group continued on LG diet during the post-CGS induction period when compared with normal control group. In garlic or onion diet–fed groups, the same was not much different from the basal control diet–fed animals. Serum phospholipid of CGS-induced mice remained lowered when they were maintained on either LG diet or basal control diet. Garlic- or onion-containing diets, on the other hand, significantly restored serum phospholipids (15%-65% increase in 5 weeks and 17%-38% increase in 10 weeks compared with basal control group). As a result of this beneficial modification of both cholesterol and phospholipid concentrations, cholesterol to phospholipid ratio was decreased by 18% to 44% in these animals compared with basal control group after 10 weeks. It is significant to note that control diet (C) feeding during post-CGS induction did not beneficially restore alterations in lipid parameters in serum, whereas dietary *Allium* spices brought about restoration of the same to a significant extent.

3.3. Effect of feeding garlic and onion for 5 and 10 weeks on liver lipid profile in CGS-prevailing mice

Liver cholesterol in mice continued on LG diet during post-CGS induction period was increased by 2.4 and 2.8 times compared with normal controls at 5 and 10 weeks, respectively (Table 3). Groups of mice fed garlic- or onion-containing diets during post-CGS induction period had lesser hepatic cholesterol as compared with basal control group. The decreases brought about by these *Allium* spices were 12% to 31% at 5 weeks and 6% to 24% at 10 weeks. Similarly, hepatic triglyceride was decreased by dietary garlic and onion by 10% to 25% at 5 weeks. On the other hand, dietary garlic and onion increased the hepatic phospholipid content by 15% to 32% after feeding for 10 weeks when compared with the animals maintained on basal control diet, whereas there was no significant alteration in 5 weeks. The cholesterol to phospholipid ratio was increased in LG group by 3.65-fold, indicating increased cholesterol content in liver compared with controls. In garlic- and onion-fed groups, cholesterol to phospholipid ratio was decreased by 14% to 38% after 5 weeks and by 16% to 43% after 10 weeks compared with basal control group. These results indicated that garlic and onion could effectively decrease liver cholesterol and elevate phospholipid levels, resulting in a decreased cholesterol to phospholipid ratio under CGS-prevailing conditions. It is important to note here that control diet (C) feeding during post-CGS induction did not significantly restore the altered hepatic lipid profile.

3.4. Effect of feeding garlic and onion for 5 and 10 weeks on biliary lipid profile in CGS-prevailing mice

Biliary lipid profile of animals maintained for 5 and 10 weeks on various test diets during post-CGS induction is

Table 2
Effect of feeding garlic and onion for 5 and 10 weeks on serum lipid profile in CGS-prevailing mice

Diet group	Total cholesterol		Phospholipid		Triglycerides		C/PL	
	5 wk	10 wk	5 wk	10 wk	5 wk	10 wk	5 wk	10 wk
Normal control[a]	195.4 ± 9.20	181.9 ± 14.9	323.6 ± 19.3	316.4 ± 6.70	157.9 ± 16.9	156.5 ± 8.04	0.60 ±0.04	0.57 ± 0.04
LG	470.8 ± 21.5*	480.3 ± 19.0*	164.5 ± 9.64*	200.3 ± 8.48*	195.6 ± 12.2*	174.3 ± 8.51*	2.86 ± 0.31*	2.40 ± 0.18*
C	454.6 ± 14.8	445.1 ± 18.9	177.8 ± 14.3	216.5 ± 7.80	186.5 ± 17.7	169.8 ± 6.82	2.56 ± 0.15	2.05 ± 0.16
C + garlic (R)	432.8 ± 15.0	347.2 ± 28.8†	274.3 ± 12.0†	282.9 ± 12.4†	168.4 ± 15.4	158.8 ± 7.21	1.58 ± 0.13†	1.23 ± 0.13†
C + garlic (H)	441.3 ± 14.6	426.2 ± 17.8	205.2 ± 17.0†	252.7 ± 19.8†	182.1 ± 14.7	158.4 ± 7.80	2.19 ± 0.31†	1.69 ± 0.15†
C + onion (R)	431.0 ± 11.8	400.5 ± 14.6†	237.9 ± 19.0†	254.0 ± 11.8†	172.8 ± 6.82	162.8 ± 6.10	1.81 ± 0.14†	1.58 ± 0.17†
C + onion (H)	415.4 ± 13.3	345.7 ± 23.3†	292.8 ± 19.1†	298.9 ± 19.6†	177.1 ± 14.6	160.1 ± 4.90	1.14 ± 0.07†	1.15 ± 0.06†

Values expressed as milligrams per deciliter are mean ± SD of 18 mice per group. Animals in all diet groups except normal control were fed initially with LG diet for 10 weeks to induce CGS. C/PL indicates cholesterol to phospholipid ratio.

[a] Animals in normal control group were maintained on basal control diet throughout.

* Statistically significant when compared with normal control group at $P < .01$.

† Statistically significant when compared with basal control group at $P < .01$.

Table 3
Effect of feeding garlic and onion for 5 and 10 weeks on liver lipid profile in CGS-prevailing mice

Diet group	Cholesterol		Phospholipid		Triglycerides		C/PL	
	5 wk	10 wk	5 wk	10 wk	5 wk	10 wk	5 wk	10 wk
Normal control[a]	9.77 ± 1.11	9.10 ± 1.75	47.4 ± 5.46	47.9 ± 5.62	31.5 ± 5.83	31.0 ± 4.56	0.20 ± 0.03	0.19 ± 0.04
LG	26.0 ± 4.46*	25.7 ± 2.70*	36.2 ± 4.62*	36.0 ± 4.17*	57.9 ± 4.14*	52.4 ± 4.75*	0.73 ± 0.16*	0.72 ± 0.15*
C	24.1 ± 2.56	21.1 ± 2.26	36.1 ± 2.35	34.1 ± 2.55	54.1 ± 3.21	49.9 ± 3.21	0.66 ± 0.13	0.61 ± 0.13
C + garlic (R)	17.9 ± 2.94†	16.9 ± 3.13†	39.8 ± 3.42	39.3 ± 3.48†	45.4 ± 2.82†	44.7 ± 3.10	0.45 ± 0.08†	0.43 ± 0.09†
C + garlic (H)	21.2 ± 1.81	19.8 ± 1.40	37.8 ± 5.37	39.5 ± 5.22†	48.7 ± 4.71	49.4 ± 5.15	0.57 ± 0.10†	0.51 ± 0.10†
C + onion (R)	20.0 ± 2.58†	17.9 ± 2.39†	37.4 ± 3.80	40.7 ± 4.23†	46.3 ± 3.72†	47.3 ± 4.09	0.53 ± 0.06†	0.44 ± 0.09†
C + onion (H)	16.6 ± 2.96†	16.0 ± 2.57†	40.4 ± 3.82	45.0 ± 2.15†	40.6 ± 4.78†	44.3 ± 6.72	0.41 ± 0.08†	0.35 ± 0.06†

Values given as milligrams per gram fresh liver are mean ± SD of 18 mice per group. Animals in all diet groups except normal control were fed initially with LG diet for 10 weeks to induce CGS.

[a] Animals in normal control group were maintained on basal control diet throughout.

* Statistically significant when compared with normal control group at $P < .01$.

† Statistically significant when compared with basal control group at $P < .01$.

presented in Table 4. Cholesterol content of bile was increased by 6.34- and 5.7-fold in LG diet group compared with normal control group when measured at 5- and 10-week intervals. On the other hand, when compared with the animals maintained on basal control diet during post-CGS induction period, biliary cholesterol content was decreased by 42% and 25% by dietary garlic (raw and heat processed) and by 35% and 51% by dietary onion (raw and heat processed) after 5 weeks. This decrease in biliary cholesterol caused by dietary garlic (44% and 28%) or dietary onion (36% and 55%) was similar after 10 weeks. Biliary bile acid content remained essentially unaltered by the treatment with either garlic or onion as compared with control animals. Similarly, biliary phospholipid was not significantly altered in the test groups during post-CGS induction. The cholesterol to phospholipid ratio in the bile of LG group was much higher in view of the increased cholesterol content (Fig. 3). On the other hand, this ratio in garlic and onion diet groups was 29% to 40% lower than basal control group in 5 weeks and 30% to 46% lower than basal control group in 10 weeks. Similarly, cholesterol to bile acid ratio in bile was higher (0.19) in LG group, whereas it was significantly lower than basal control group upon feeding garlic and onion during post-CGS induction (Fig. 3).

The CSI in the bile was 2.1 in animals maintained on LG group during post-CGS induction period (Fig. 4). The CSI of bile was prominently lowered when the animals were maintained on garlic or onion diets during the same period (0.94 to 1.26 at 5 weeks and 0.91 to 1.13 at 10 weeks). The HI of bile was 0.053 in the animals maintained on LG diet and 0.045 in those maintained on basal control diet during the post-CGS induction period (Fig. 4). The HI of bile was decreased to −0.07 and −0.02 in the case of dietary garlic and −0.05 and −0.10 in the case of dietary onion after 5 weeks (Fig. 4). The same was similarly decreased by dietary garlic and onion fed for 10 weeks.

The content of hydrophilic bile acids tauromuricholic acid and tauroursodeoxycholic acid were decreased in animals maintained on LG diet, whereas they were increased by feeding the 2 test spices (Tables 5 and 6). On the other hand, hydrophobic bile acids taurocholic acid and taurodeoxycholic

Table 4
Effect of feeding garlic and onion for 5 and 10 weeks on biliary lipids in CGS-prevailing mice

Dietary group	Biliary lipids (mmol/L)							
	Cholesterol		Phospholipid		Bile acids		Total lipid (g/dL)	
	5 wk	10 wk	5 wk	10 wk	5 wk	10 wk	5 wk	0 wk
Normal control[a]	5.25 ± 0.25	5.54 ± 0.46	12.7 ± 1.52	13.8 ± 1.33	200.7 ± 12.3	200.4 ± 8.05	11.0 ± 1.12	11.1 ± 0.54
LG	33.3 ± 2.06*	31.6 ± 1.85*	22.2 ± 2.29*	24.1 ± 1.62*	172.8 ± 16.1*	186.8 ± 8.15*	11.5 ± 0.85	12.3 ± 0.62
C	30.1 ± 3.01	26.5 ± 3.01	20.9 ± 1.78	21.9 ± 1.44	169.8 ± 12.3	175.1 ± 10.3	11.5 ± 0.35	11.4 ± 0.15
C + garlic (R)	17.6 ± 1.77†	14.9 ± 1.54†	19.3 ± 3.11	19.7 ± 1.76	182.9 ± 20.6	191.9 ± 9.00†	11.2 ± 1.15	11.6 ± 0.59
C + garlic (H)	22.6 ± 3.60†	19.0 ± 0.77†	23.1 ± 2.72	22.7 ± 2.07	180.1 ± 11.0	198.0 ± 8.58†	11.5 ± 0.58	11.7 ± 0.39
C + onion (R)	19.6 ± 1.33†	16.9 ± 1.01†	21.6 ± 2.57	20.2 ± 1.53	178.5 ± 20.2	193.3 ± 5.94†	11.2 ± 1.13	1.7 ± 0.18
C + onion (H)	4.7 ± 0.93†	11.8 ± 0.55†	16.9 ± 1.91†	18.0 ± 1.95†	196.7 ± 19.5†	199.5 ± 11.3†	11.5 ± 0.90	11.6 ± 0.50

Values given as milligrams per gram fresh liver are mean ± SD of 18 mice per group. Animals in all diet groups except normal control were fed initially with LG diet for 10 weeks to induce CGS.

[a] Animals in normal control group were maintained on basal control diet throughout.

* Statistically significant when compared with normal control group at $P < .01$.

† Statistically significant when compared with basal control group at $P < .01$.

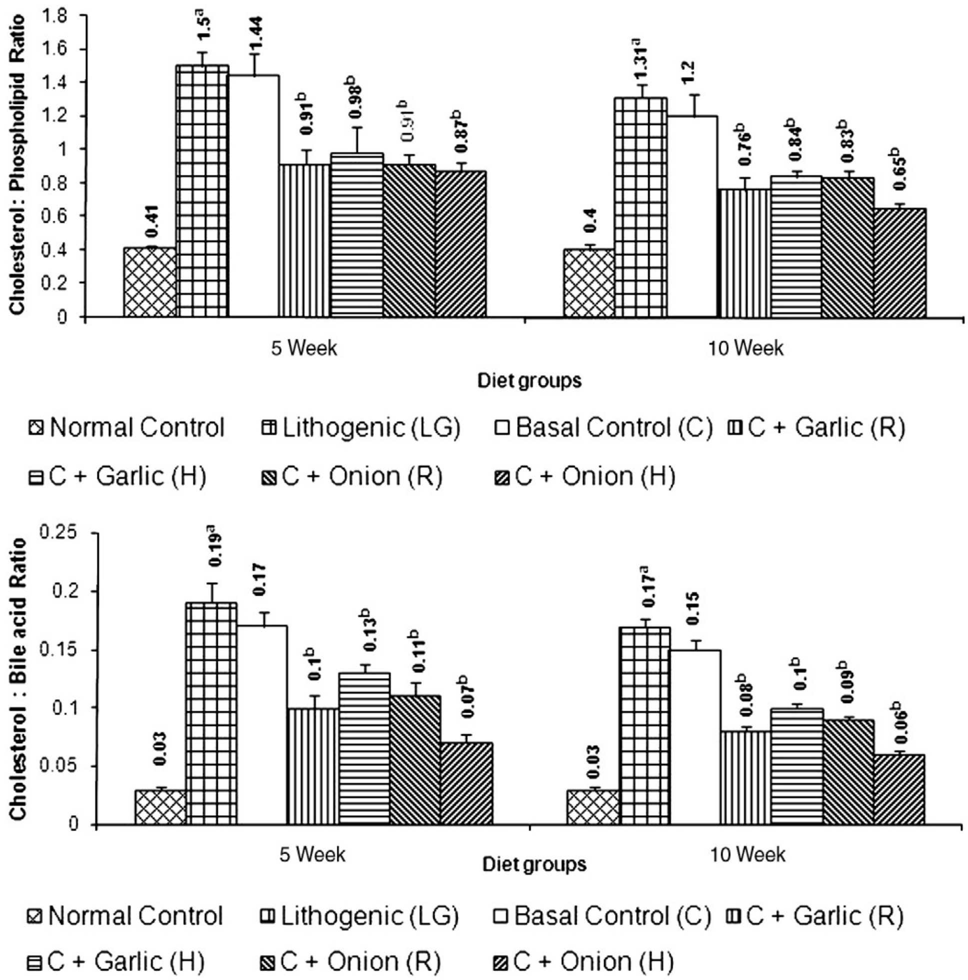

Fig. 3. Effect of feeding of garlic and onion on cholesterol to phospholipid ratio and cholesterol to bile acid ratio in the bile in CGS-prevailing mice. Values are mean of 6 samples per group, each sample constituting 3 mice. Animals in all diet groups except normal control were fed initially with LG diet for 10 weeks to induce CGS. Animals in normal control group were maintained on basal control diet throughout. All values in LG group were statistically different ($P < .01$) when compared with normal control group that was maintained on basal control diet throughout. All values in garlic- and onion-fed groups were statistically different when compared with C group ($P < .01$).

acid were increased by LG diet-fed mice, whereas these were decreased by dietary garlic and onion. The biliary phospholipid fatty acid profile shown in Tables 7 and 8 indicated that palmitic acid content was considerably reduced by dietary spice treatment during post-CGS induction period (by 20%-33% at 5 weeks and by 11%-29% at 10 weeks). This was accompanied by an increase in oleic acid content especially in onion treatment (Tables 7 and 8).

3.5. *Effect of feeding garlic and onion for 5 and 10 weeks on sterol-metabolizing enzymes in the liver of CGS-prevailing mice*

Hydroxymethylglutaryl–CoA reductase is the rate-limiting enzyme in cholesterol biosynthesis. Continuation of the feeding of LG diet during post-CGS induction significantly decreased hepatic HMG-CoA reductase activity (Table 9). Similarly, enzymes involved in the biosynthesis of bile acids from cholesterol, namely, cholesterol-7α-hydroxylase and sterol-27-hydroxylase, were decreased during LG diet feeding. Feeding of garlic- or onion-containing diets during post-CGS induction increased the activity of hepatic HMG-CoA reductase by 1.6- to 2.7-fold after 5 weeks and by 1.9- to 3.4-fold compared with basal control diet. Similarly, feeding of these *Allium* spices increased the activity of cholesterol-7α-hydroxylase (by 34%, 0%, 16%, and 53% after 5 weeks and by 23%, 0%, 11%, and 51% after 10 weeks, respectively) compared with basal control group (C). Similarly, dietary garlic and onion increased the activity of hepatic sterol-27-hydroxylase activity (by 23% and 33% by dietary raw and heat-processed onion after 5 weeks and by 31%, 10%, 34%, and 64%, respectively, in raw and heat-processed garlic and onion groups) compared with basal control group. These results indicated that dietary *Allium* spices effectively increased the activity of sterol-metabolizing enzymes in CGS-prevailing conditions in mice.

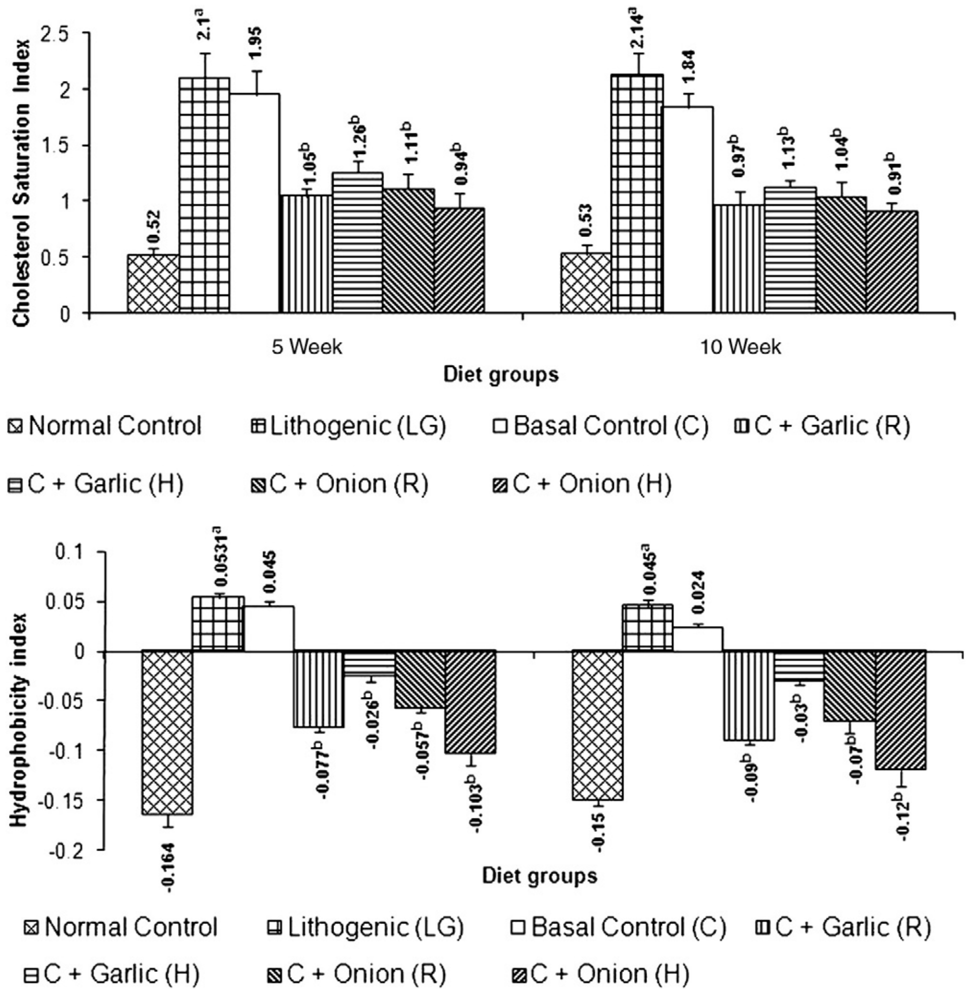

Fig. 4. Effect of feeding garlic and onion on CSI and HI of bile CGS-prevailing mice. Values are mean ± SD of 6 samples per group, each sample constituting 3 mice. Animals in all diet groups except normal control were fed initially with LG diet for 10 weeks to induce CGS. Animals in normal control group were maintained on basal control diet throughout. All values in LG group were statistically different ($P < .01$) when compared with normal control group that was maintained on basal control diet throughout. All values in garlic- and onion-fed groups were statistically different when compared with C group ($P < .01$).

The results of this investigation indicated that dietary *Allium* spices could significantly decrease cholesterol and increase bile acids and phospholipids in gallbladder bile in CGS-prevailing conditions, thus effectively reversing the increased cholesterol to phospholipid and cholesterol to bile acid ratios in gallbladder bile in CGS-prevailing conditions.

Table 5
Effect of feeding garlic and onion for 5 weeks on various bile salts in CGS-prevailing mice

Diet group	TMC	TUDC	TC	TCDC	TDC	GC
Normal control[a]	0.27 ± 0.040	0.020 ± 0.001	0.59 ± 0.004	0.04 ± 0.001	0.05 ± 0.003	0.02 ± 0.001
LG	0.05 ± 0.004*	0.008 ± 0.0005*	0.74 ± 0.006*	0.04 ± 0.002	0.12 ± 0.002*	0.02 ± 0.001
C	0.10 ± 0.005	0.006 ± 0.0005	0.70 ± 0.005	0.04 ± 0.002	0.10 ± 0.002	0.02 ± 0.001
C + garlic (R)	0.19 ± 0.004†	0.012 ± 0.006†	0.64 ± 0.008†	0.04 ± 0.002	0.08 ± 0.004†	0.02 ± 0.001
C + garlic (H)	0.14 ± 0.004†	0.013 ± 0.001†	0.63 ± 0.015†	0.05 ± 0.007†	0.11 ± 0.005	0.022 ± 0.001
C + onion (R)	0.19 ± 0.001†	0.013 ± 0.001†	0.58 ± 0.014†	0.051 ± 0.006†	0.12 ± 0.009†	0.026 ± 0.005†
C + onion (H)	0.24 ± 0.022†	0.013 ± 0.002†	0.57 ± 0.016†	0.044 ± 0.003	0.11 ± 0.02	0.018 ± 0.001

Values are mean ± SD of 6 samples per group, each sample constituting 3 mice. Animals in all diet groups except normal control were fed initially with LG diet for 10 weeks to induce CGS. TMC indicates tauromuricholic acid; TUDC, tauroursodeoxycholic acid; TC, taurocholic acid; TCDC, taurochenodeoxycholic acid; TDC, taurodeoxycholic acid; GC, glycocholic acid.

[a] Animals in normal control group were maintained on basal control diet throughout.

* Statistically significant when compared with normal control group at $P < .01$.

† Statistically significant when compared with basal control group at $P < .01$.

Table 6
Effect of feeding garlic and onion for 10 weeks on various bile salts in CGS-prevailing mice

Diet group	TMC	TUDC	TC	TCDC	TDC	GC
Normal control[a]	0.25 ± 0.020	0.020 ± 0.003	0.59 ± 0.05	0.046 ± 0.006	0.05 ± 0.002	0.02 ± 0.002
LG	0.05 ± 0.006*	0.009 ± 0.004*	0.73 ± 0.03*	0.040 ± 0.004	0.12 ± 0.003*	0.02 ± 0.003
C	0.15 ± 0.004	0.014 ± 0.004	0.69 ± 0.02	0.040 ± 0.004	0.09 ± 0.003	0.02 ± 0.003
C + garlic (R)	0.20 ± 0.006[†]	0.018 ± 0.004[†]	0.63 ± 0.007[†]	0.030 ± 0.003[†]	0.08 ± 0.007	0.02 ± 0.003
C + garlic (H)	0.16 ± 0.004	0.016 ± 0.002	0.63 ± 0.01[†]	0.050 ± 0.010[†]	0.12 ± 0.020	0.02 ± 0.001
C + onion (R)	0.20 ± 0.007[†]	0.013 ± 0.001	0.61 ± 0.02[†]	0.035 ± 0.009[†]	0.11 ± 0.009[†]	0.027 ± 0.003[†]
C + onion (H)	0.26 ± 0.028[†]	0.015 ± 0.008	0.54 ± 0.02[†]	0.043 ± 0.004	0.11 ± 0.020[†]	0.019 ± 0.002

Values (given as mole fraction) are mean ± SD of 6 samples per group, each sample constituting 3 mice. Animals in all diet groups except normal control were fed initially with LG diet for 10 weeks to induce CGS.

[a] Animals in normal control group were maintained on basal control diet throughout.

* Statistically significant when compared with normal control group at $P < .01$.

[†] Statistically significant when compared with basal control group at $P < .01$.

It is to be noted that control diet feeding after CGS formation did not alter these lipid parameters to any significant extent.

4. Discussion

In pathologic conditions, when the liver secretes higher amounts of cholesterol and lower amounts of bile acids and phospholipids into the bile, the cholesterol to phospholipid and cholesterol to bile acid ratios increase in bile. These events lead to supersaturation of bile, resulting in the nucleation of cholesterol crystals. In the presence of continuous supply of cholesterol, the crystal grows into a big stone leading to the formation of CGS. Removal of CGS is usually carried out by either large doses of cholelitholytic drugs or by cholecystectomy [30]. Oral litholysis with ursodeoxycholic acid might still be appropriate for patients who are unfit for surgery. But the major drawback of these 2 treatments is that the stones reappear upon discontinuation of the drug or after a lapse of time following lithotripsy [15]. A better alternative to address CGS would be dietary intervention, which could help in the prevention of incidence, regression of existing CGS, and stopping the possible recurrence. Data on the effects of dietary components on gallbladder bile in patients and in animals with established cholelithiasis are very limited.

To study the hypothesis that dietary hypocholesterolemic constituents can regress preformed CGS, this investigation was conducted by inducing CGS in gallbladder of mice by feeding an LG diet and subsequently evaluating its regression under spice feeding. Cholesterol gallstone was induced in 100% of the mice by feeding LG diet for 10 weeks. After confirming the formation of CGS, LG diet was discontinued; and they were fed either basal control or various experimental diets containing garlic or onion for 5 and 10 weeks. The results of the present investigation showed that feeding garlic and onion for 5 weeks after the induction of CGS in mice was sufficient to cause regression of CGS up to 27% and that a longer 10-week duration of feeding of these spices caused 47% regression. Substitution of LG diet with basal control diet after CGS induction in mice neither caused any marked regression of CGS nor reduced the CSI and restored the various lipid parameters in the bile. It is clear that mere withdrawal of LG diet could not regress preformed CGS in the mice, whereas feeding *Allium* spices could influence both biliary lipid metabolism and CGS pathogenesis in particular.

The antilithogenic potential of these 2 *Allium* spices is closely associated with their hypocholesterolemic property. The decrease in serum cholesterol by dietary garlic or onion after the stopping of l0-week LG diet regimen was more evident at the end of 10 weeks of post-CGS induction period.

Table 7
Effect of feeding garlic and onion for 5 weeks on biliary fatty acid composition in CGS-prevailing mice

Diet group	16:0	16:1	18:0	18:1	18:2	20:4
Normal control[a]	18.9 ± 1.25	4.98 ± 0.85	3.72 ± 0.54	63.5 ± 8.52	6.89 ± 0.58	1.96 ± 0.11
LG	29.0 ± 3.20*	4.86 ± 0.89	3.56 ± 0.25	49.9 ± 6.5*	9.27 ± 0.39*	3.54 ± 0.12*
C	29.6 ± 2.22	4.77 ± 0.81	3.64 ± 0.21	53.6 ± 5.3	10.7 ± 0.29	2.24 ± 0.11
C + garlic (R)	23.8 ± 2.74[†]	3.74 ± 1.23[†]	5.41 ± 1.20[†]	53.6 ± 3.45	8.40 ± 1.52[†]	1.98 ± 0.19
C + garlic (H)	23.2 ± 2.22[†]	3.10 ± 0.25[†]	1.68 ± 0.23[†]	52.4 ± 5.42	11.6 ± 2.30	7.99 ± 0.36[†]
C + onion (R)	21.2 ± 2.36[†]	3.56 ± 0.53[†]	4.36 ± 1.22[†]	55.2 ± 6.21	8.65 ± 1.89[†]	6.82 ± 0.17[†]
C + onion (H)	19.7 ± 1.89[†]	2.83 ± 0.16[†]	3.96 ± 0.92	58.1 ± 3.49	8.21 ± 0.87[†]	7.21 ± 0.31[†]

Values (given as percentage total fatty acids) are mean ± SD of 6 samples per group, each sample constituting 3 mice. Animals in all diet groups except normal control were fed initially with LG diet for 10 weeks to induce CGS.

[a] Animals in normal control group were maintained on basal control diet throughout.

* Statistically significant when compared with normal control group at $P < .01$.

[†] Statistically significant when compared with basal control group at $P < .01$.

Table 8
Effect of feeding garlic and onion for 10 weeks on biliary fatty acid composition in CGS-prevailing mice

Diet group	16:0	16:1	18:0	18:1	18:2	20:4
Normal control[a]	19.85 ± 1.02	4.69 ± 0.12	3.12 ± 0.32	63.59 ± 3.10	6.79 ± 0.56	1.93 ± 0.20
LG	30.28 ± 2.31*	4.49 ± 0.23	3.59 ± 0.34*	48.82 ± 3.21*	9.85 ± 0.45*	3.04 ± 0.13*
C	28.56 ± 1.51	4.41 ± 0.13	3.16 ± 0.24	52.36 ± 2.51	10.55 ± 0.54	2.41 ± 0.32
C + garlic (R)	22.98 ± 2.05†	7.12 ± 0.25†	5.91 ± 0.45†	53.26 ± 3.56	8.56 ± 0.98†	2.18 ± 0.30
C + garlic (H)	25.56 ± 1.36†	3.54 ± 0.31†	1.54 ± 0.21†	50.00 ± 4.10	11.62 ±1.54	7.99 ± 0.56†
C + onion (R)	21.21 ± 2.45†	3.56 ± 0.13†	4.36 ± 0.42†	54.04 ± 2.30	11.35 ± 1.64	5.48 ± 0.41†
C + onion (H)	20.18 ± 1.44†	3.05 ± 0.21†	3.53 ± 0.31	56.98 ± 3.32	9.54 ± 0.87	6.72 ± 0.32†

Values (given as percentage total fatty acids) are mean ± SD of 6 samples per group, each sample constituting 3 mice. Animals in all diet groups except normal control were fed initially with LG diet for 10 weeks to induce CGS.

[a] Animals in normal control group were maintained on basal control diet throughout.

* Statistically significant when compared with normal control group at $P < .01$.

† Statistically significant when compared with basal control group at $P < .01$.

Such a hypocholesterolemic effect was also accompanied by a simultaneous increase in serum phospholipid content. The observed decreased activity of hepatic HMG-CoA reductase in LG (high cholesterol) diet feeding is due to the negative feedback mechanism. In animals fed basal diet containing either garlic or onion for 5 or 10 weeks subsequent to the induction of CGS, the absence of LG has resulted in the observed reversal of the suppression of this enzyme activity. Increased activity of hepatic cholesterol-7α-hydroxylase by the *Allium* spices is probably the mechanism for facilitating cholesterol metabolism and excretion. It has been suggested that any increase in the activity of this enzyme is invariably paralleled by similar increases in the activity of hepatic HMG-CoA reductase as a compensatory mechanism. That is why we have observed parallel increases in the activities of these 2 regulatory enzymes of cholesterol metabolism during dietary treatment of garlic or onion.

It has been reported that dietary curcumin of turmeric, capsaicin of red pepper, and fenugreek seeds significantly regressed the preestablished CGS in experimental mice [8,9]. Similarly, feeding fish oil decreased the CSI by 25% after 5 weeks of treatment of cholelithiasis-prevailing patients [31]. On the other hand, dietary supplementation of linoleic acid for 3 weeks did not alter the biliary lipid composition or CSI [31].

Feeding of garlic or onion resulted in a decrease in major part of the hepatic cholesterol pool, which is to be secreted into bile by its conversion to bile acids resulting in an increased bile acid concentration. Phospholipid concentration was also increased upon feeding these *Allium* spices, causing a phospholipid-rich system. Increased bile acid and phospholipid concentrations promote solubilization of the biliary cholesterol in mixed micelles, which are stable, hence reducing the possibility of nucleation of cholesterol crystals [32]. Cholesterol crystal inhibiting factors such as proteins present in the bile have been reported by many workers [33-36]. These events affect the dynamic equilibrium of bile in such a way that the cholesterol supply to the growing CGS is cut off, and the CGS may degenerate or regress in the absence of continued supply of cholesterol and by the action of antinucleation proteins. Spices reduce the total cholesterol pool, which is evident from the lowered cholesterol to phospholipid ratio in serum and liver. The current animal study demonstrates that *Allium* spices have a profound effect on the lipid profile in blood, liver, and bile. Considering the above facts, it can be concluded that a consistent exchange

Table 9
Effect of feeding garlic and onion for 5 and 10 weeks on sterol-metabolizing enzymes in liver of CGS-prevailing mice

Diet group	HMG-CoA reductase[b]		Cholesterol-7α-hydroxylase[c]		Sterol-27-hydroxylase[c]	
	5 wk	10 wk	5 wk	10 wk	5 wk	10 wk
Normal control[a]	57.6 ± 5.62	54.3 ± 2.38	28.5 ± 1.62	31.6 ± 1.28	63.5 ± 2.68	73.2 ± 3.56
LG	14.6 ± 2.06*	9.29 ± 1.97*	9.63 ± 1.38*	8.45 ± 1.26*	26.8 ± 1.39*	22.8 ± 1.39*
C	19.9 ± 2.65	22.4 ± 2.95	11.6 ± 1.35	14.2 ± 1.33	31.2 ± 2.14	30.2 ± 2.11
C + garlic (R)	69.3 ± 4.81†	86.4 ± 3.49†	15.6 ± 1.93†	17.5 ± 1.36†	32.7 ± 2.59	39.6 ± 3.41†
C + garlic (H)	52.6 ± 5.65†	64.8 ± 2.83†	11.4 ± 1.06	13.2 ± 1.69	30.8 ± 4.13	33.2 ± 2.62
C + onion (R)	58.2 ± 4.82†	67.3 ± 2.31†	13.4 ± 1.94	15.7 ± 1.68	38.4 ± 1.76†	40.4 ± 3.98†
C + onion (H)	73.6 ± 5.40†	98.4 ± 4.18†	17.8 ± 1.63†	21.4 ± 2.69†	41.6 ± 3.65†	49.6 ± 2.49†

Values are mean ± SD of 6 samples, each sample representing 3 mice. Animals in all diet groups except normal control were fed initially with LG diet for 10 weeks to induce CGS.

[a] Animals in normal control group were maintained on basal control diet throughout.

[b] Nanomoles of CoA formed per minute per milligram protein.

[c] Picomoles of 7α-hydroxycholesterol and 27-hydroxycholesterol formed per minute per milligram protein.

* Statistically significant when compared with normal control group at $P < .01$.

† Statistically significant when compared with basal control group at $P < .01$.

and redistribution of biliary lipids between the carriers occur, which is probably mediated by protein, thus inhibiting CGS formation or promoting the regression of CGS. The current investigation which has documented the potential of dietary garlic and onion in accelerating the regression of preformed CGS in experimental animals lends further support to the health beneficial antilithogenic influence of these *Allium* spices observed by us in terms of reducing the incidence of CGS during experimental induction with LG [14]. This finding assumes importance in the context of evolving an alternative to address CGS by dietary intervention, which could help in the prevention of incidence, regression of the existing CGS, and stopping of the possible recurrence.

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References

[1] Apstein MD, Carey MC. Pathogenesis of cholesterol gallstones a parsimonious hypothesis. Eur J Clin Invest 1996;26:343-52.
[2] Marzolo MP, Rigotti A, Nervi F. Secretion of biliary lipids from the hepatocyte. Hepatology 1990;12(Suppl):134-42.
[3] Von Erpecum KJ, Von Berge-Henegouwen GP. Pathogenic factors in cholesterol gallstone disease. Scand J Gastroenterol 1989;24 (Suppl):83-90.
[4] Thornton JR, Emmett PM, Heaton KW. Diet and gallstone effect on refined and unrefined carbohydrate diets on bile cholesterol saturation and bile acid metabolism. Gut 1983;24:2-6.
[5] Ebihara K, Kiriyama S. Prevention of CGS by a water soluble fibre–Konjac Mannan in mice. Nutr Rep Int 1985;32:223-9.
[6] Mott GE, Jackson EM, McMohan CA. Effect of dietary cholesterol, type of fat and sex on bile lipid composition of adult baboons. Am J Clin Nutr 1992;56:511-5.
[7] Srinivasan K, Sambaiah K, Chandrasekhara N. Spices as beneficial hypocholesterolemic food adjuncts: a review. Food Rev Int 2004;20: 187-220.
[8] Hussain MS, Chandrasekhara N. Influence of curcumin and capsaicin on cholesterol gallstone induction in hamsters and mice. Nutr Res 1993;13:349-57.
[9] Reddy RLR, Srinivasan K. Dietary fenugreek seed regresses preestablished cholesterol gallstones in experimental mice. Can J Physiol Pharmacol 2009;87:684-93.
[10] Hussain MS, Chandrasekhara N. Effect of curcumin and capsaicin on the regression of pre-established CGS in mice. Nutr Res 1994;14:1561-74.
[11] Kamanna VS, Chandrasekhara N. Effect of garlic (*Allium sativum* Linn.) on serum lipoprotein and lipoprotein cholesterol levels in albino rats rendered hypercholesterolemic by feeding cholesterol. Lipids 1982;17:483-8.
[12] Sambaiah K, Srinivasan K. Secretion and composition of bile in rats fed diets containing spices. J Food Sci Technol 1991;28:35-8.
[13] Platel K, Srinivasan K. Stimulatory influence of select spices on bile secretion in rats. Nutr Res 2000;20:1493-503.
[14] Vidyashankar S, Sambaiah K, Srinivasan K. Dietary garlic and onion reduces the incidence of atherogenic diet induced cholesterol gallstones in experimental mice. Br J Nutr 2009;101:1621-9.
[15] Bouchier AD. New drugs for gallstones. Br Med J 1990;300:5927-31.
[16] Akiyoshi T, Uchida K, Takase H, Nomura Y, Takeuchi N. Cholesterol gallstones in alloxan-diabetic mice. J Lipid Res 1986;27:915-24.
[17] Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 1959;37:911-7.
[18] Turley SD, Dietschy JM. Reevaluation of 3α-hydroxysteroid dehydrogenase assay for total bile acids. J Lipid Res 1970;19:924-8.
[19] Folch J, Lees M, Sloane-Stanley GH. A simple method for the isolation and purification of total lipids from animal tissue. J Biol Chem 1957;226:497-509.
[20] Searcy RL, Bergquist LM. A new color reaction for the quantification of serum cholesterol. Clin Chim Acta 1960;5:192-9.
[21] Stewart JCM. Colorimetric estimation of phospholipids with ammonium ferrothiocyanate. Anal Biochem 1980;104:10-4.
[22] Fletcher MJ. A colorimetric method for estimating serum triglycerides. Clin Chim Acta 1968;22:303-7.
[23] Rossi SS, Converse JL, Hofmann AF. High pressure liquid chromatographic analysis of conjugated bile acids in human bile: simultaneous resolution of sulfated and unsulfated lithocholyl amidates and common conjugated bile acids. J Lipid Res 1987;28: 589-95.
[24] Heuman DM. Quantitative estimation of the hydrophilic: hydrophobic balance of mixed bile salt solutions. J Lipid Res 1989;30:719-30.
[25] Carey MC. Critical tables for calculating the cholesterol saturation of native bile. J Lipid Res 1978;19:945-55.
[26] Morrison MR, Smith M. Preparation of fatty acid methyl esters and dimethyl acetyls from lipids with boron trifluoride methanol. J Lipid Res 1964;5:600-8.
[27] Hulcher FH, Oleson WH. Simplified spectrophotometric assay for microsomal 3-hydroxy-3-methylglutaryl coenzyme-A reductase by measurement of coenzyme-A. J Lipid Res 1973;14:625-31.
[28] Petrack B, Latario BJ. Synthesis of 27-hydroxycholesterol in rat liver mitochondria: HPLC assay and marked activation by exogenous cholesterol. J Lipid Res 1993;34:643-9.
[29] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265-75.
[30] Roslyn IT, Binns GS, Mughes BF. Open cholecystectomy. A contemporary analysis of 42,474 patients. Ann Surg 1993;218:129-37.
[31] Berr F, Holl J, Jungst D, Fischer S, Richter WO, Seifferth B, et al. Dietary n-3 polyunsaturated fatty acids decrease biliary cholesterol saturation in gallstone disease. Hepatology 1992;16:960-7.
[32] Holan KR, Holzbach RT, Hermann RE, Cooperman AM, Claffey WJ. Nucleation time; a key factor in the pathogenesis of cholesterol gallstone disease. Gastroenterology 1979;77:611-7.
[33] Sedaghat A, Grundy SM. Cholesterol crystals and the formation of human gallstones. N Engl J Med 1980;302:1274-7.
[34] Burnstein MJ, Ilson RG, Petrunka CN, Taylor RD, Strasberg SM. Evidence for a potent nucleating factor in the gallbladder bile of patients with cholesterol gallstones. Gastroenterology 1985;85: 801-7.
[35] Gallinger S, Taylor RD, Harvey PR, Petrunka CN, Strasberg SM. Effect of mucous glycoprotein on nucleation time of human bile. Gastroenterology 1985;89:648-58.
[36] Groen AK, Stout JP, Draper JA, Hoek FJ, Grijm R, Tygat GN. Effect of cholesterol nucleation promoting activity on cholesterol solubilization in model bile. Hepatology 1988;8:347-52.