

Potential of anti-cholelithogenic influence of dietary tender cluster beans (*Cyamopsis tetragonoloba*) by garlic (*Allium sativum*) in experimental mice

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Background & objectives: Dietary fibre-rich tender cluster beans (*Cyamopsis tetragonoloba*; CB) are known to exert beneficial cholesterol lowering influence. We examined the influence of a combination of dietary tender CB and garlic (*Allium sativum*) in reducing the cholesterol gallstone formation in mice.

Methods: Cholesterol gallstones were induced in Swiss mice by feeding a high-cholesterol diet (HCD) for 10 wk. Dietary interventions were made with 10 per cent CB and 1 per cent garlic included individually or together along with HCD. A total of 100 mice were divided into five groups of 20 mice each.

Results: Dietary CB, garlic and CB+garlic reduced the formation of cholesterol gallstones by 44, 25 and 56 per cent, respectively, lowered cholesterol by 23-48, 16-24, and 24-58 in bile, serum, and liver, respectively. Cholesterol saturation index in bile and cholesterol: phospholipid ratio in circulation and hepatic tissue were significantly lowered by these dietary interventions, with highest beneficial effect from CB+garlic. Activities of hepatic cholesterol metabolizing enzymes were modulated by CB, garlic and CB+garlic. Elevation in lipid peroxides caused by HCD was also countered by these dietary interventions, the combination producing the highest effect.

Interpretation & conclusions: The results showed that the prevention of experimentally induced formation of cholesterol gallstones by dietary CB and garlic was due to decreased biliary cholesterol secretion and increased cholesterol saturation index. In addition of anti-lithogenic effect, dietary CB and garlic in combination had a beneficial antioxidant effect.

Key words Anti-cholelithogenic effect - cholesterol gallstones - cholesterol saturation index - garlic - hypocholesterolaemic effect - tender cluster beans

Cholesterol gallstone (CGS), a disorder resulting from hepatic and biliary cholesterol homeostasis assumes importance due to its wide prevalence across the globe. Cholesterol crystallization in bile is determined by the relative concentrations of cholesterol, bile acids,

and phospholipids which contribute to cholesterol saturation index. Decreased cholesterol excretion as also its increased biosynthesis and secretion into bile is likely to increase the risk of CGS. Prolonged whole gut transit time has been linked to an increased production

of deoxycholate, increased cholesterol secretion and hence cholesterol supersaturation in bile which leads to the formation of cholesterol stones in gallbladder¹.

Specific dietary constituents are known to influence CGS. Animal proteins such as casein are understood to favour CGS formation. Diet containing polyunsaturated fat (*e.g.* fish oil) causes a lower incidence of CGS in animals compared to diets with saturated fat². Fibre-rich diets are also considered useful. The beneficial anti-lithogenic role of hypocholesterolaemic spices or their bioactives such as fenugreek seeds, garlic, onion, curcumin and capsaicin have been documented³. Tender cluster beans (*Cyamopsis tetragonoloba*) are commonly consumed as vegetable in India and African countries. Because of their rich dietary fibre, cluster beans may have therapeutic value in the treatment of hypercholesterolaemia. Tender cluster bean constitutes 20 per cent guar gum on dry weight basis⁴. Guar gum is a water soluble polysaccharide made up of galactomannan molecules. Dietary guar gum is known to decrease postprandial glycaemia and insulinaemia and improves insulin sensitivity in animal diabetes models⁵. Guar gum also reduces cholesterol by multiple mechanisms, namely, interruption of enterohepatic circulation of bile acids resulting in their enhanced excretion, and inhibition of cholesterol absorption leading to increased faecal excretion; additionally, guar gum may entrap fat micelles thereby impeding fat absorption⁶. It has also been reported that dietary tender cluster beans at 12.5 per cent dietary level improved antioxidant status and showed hypocholesterolaemic effect in high cholesterol fed animals⁶.

Garlic (*Allium sativum*) has long been known for its cholesterol lowering effect³. Although many reports are available about different garlic extracts and raw garlic in lowering indigenous cholesterol, yet the mechanism of action is not clear. Dietary garlic is shown to be effective in inhibiting CGS in experimental mice³. In this present study, we explored the possible additive effect of dietary fibre-rich tender cluster beans and the spice adjunct garlic in reducing CGS formation in experimental mice.

Material & Methods

Chemicals: 3-Hydroxymethyl glutaryl coenzyme-A (HMG-CoA), glucose-6-phosphate dehydrogenase, cholesterol, bile salts, dipalmitoyl phosphatidylcholine, triolein, heparin, 3 α -hydroxy steroid dehydrogenase, nicotinamide adenine dinucleotide (NAD), nicotinamide adenine dinucleotide phosphate (NADP), standard bile

acids, 7 β -hydroxycholesterol, *t*-butyl hydroperoxide, tetramethoxy propane (TMP), 4-(2-hydroxyethyl)-1-piperazine ethane- sulphonic acid (HEPES) and alpha cellulose were purchased from Sigma-Aldrich Chemicals, St. Louis, USA. Dithiothreitol was purchased from Fluka Chemie (Buchs, Switzerland). Triethanol amine, EDTA, 2,3-dithionitrobenzoic acid, 1-chloro-2,4-dinitrobenzene (CDNB), sodium arsenite, Sodium metaperiodate, hydrazine hydrate, and solvents were obtained from SISCO Research Laboratory (Mumbai, India). All solvents used were of analytical grade. All vitamins, DL-methionine and choline chloride were from Hi-media (Mumbai, India). Tender cluster beans, garlic bulbs, refined ground nut oil and cane sugar were procured from the local market. Fresh tender cluster bean pods were freeze-dried and powdered. The powder was analyzed for dietary fibre according to the enzymatic assay procedure of Asp *et al*⁷. The yield of powder was around 100 g per kg of fresh tender cluster beans as a result of removal of moisture. Garlic bulbs were ground to a paste and then freeze-dried into a dry powder.

Animals and diets: This study was carried out in the department of Biochemistry and Nutrition, Central Food Technological Research Institute (CFTRI), Mysore, Karnataka, India, during January - March 2013 with approval from the Institutional Animal Ethics Committee. To evaluate the anti-lithogenic potential of tender cluster bean, 5-week old Swiss mice (OUTB/Swiss albino/Ind/CFTRI) weighing 26 ± 1 g raised at the experimental animal production facility were used. Mice ($n=100$) were randomly divided into five groups ($n=20$) and were housed in polypropylene cages (four animals per cage) and maintained at 25 ± 2 °C with humidity 65 ± 5 per cent with 12 h cycles of day and night. Basal diet (AIN-76) consisted of 65 per cent sucrose, 20 per cent casein, 5 per cent cellulose, 3.5 per cent AIN-76 mineral mix, 1 per cent AIN-76 vitamin mix, 0.3 per cent DL- methionine, 0.2 per cent choline chloride and 5 per cent refined peanut oil. Lithogenic diet (HCD) was made by including 0.5 per cent cholesterol and 0.125 per cent bile salts (1:1 mixture of sodium cholate and sodium deoxycholate) making it isoenergetic by varying sucrose concentration. Test diets were prepared by incorporating freeze-dried tender cluster bean (CB) powder (10 g per 100 g) or freeze-dried garlic (1 g per 100g) or their combination in the lithogenic diet at the expense of sucrose. These dietary levels of CB and garlic powders were fixed based on the successful cholesterol lowering effect

observed in HCD induced hypercholesterolaemic situation in rats earlier⁶. These five groups of animals, viz., basal control, HCD control, CB (10%), garlic (1%) and CB+garlic (10% + 1%) had free access to respective diets and water for a duration of 10 wk. Animal weights were recorded every week till the end of the experiment.

Gallstone scoring: At the end of feeding regimen (10 wk), the animals were fasted overnight and anaesthetized with ether. Blood was drawn by cardiac puncture and serum was separated. Gall bladders and liver were excised. Gall bladders of different groups of animals were observed for presence of CGS and severity. Gallstone scoring was done on a 5-point scale⁸. The gall bladders were punctured and volume of bile was recorded along with gallstones. The bile from the gall bladders was pooled and stored at -20 °C.

Lipid analysis: Biliary lipids were extracted by the procedure of Bligh and Dyer⁹ and methanolic phase was used to estimate the bile acid¹⁰. Chloroform phase was considered for the estimation of cholesterol and phospholipids. Lipids from serum and liver were extracted¹¹ and cholesterol was quantified¹². The HDL- and LDL-cholesterol, phospholipids, and triglycerides were quantitated according to the methods described earlier¹³⁻¹⁵.

Antioxidant molecules and lipid peroxides in liver: Hepatic glutathione was estimated by the procedure of Beutler *et al*¹⁶. Ascorbic acid was determined by estimating the 2,4-dinitrophenyl-hydrazone derivative of dehydroascorbic acid¹⁷. Lipid peroxides in liver were assayed by measuring the malondialdehyde (MDA) concentration as thiobarbituric acid reactive substances (TBARS) spectrophotometrically¹⁸.

Antioxidant enzymes in plasma and liver: Glutathione-S-transferase activity was assayed by measuring the conjugate of glutathione with CDNB used as substrate as described by Warholm *et al*¹⁹. Glutathione reductase activity was assayed by measuring according to Carlberg and Mannervik²⁰. Activities of glutathione peroxidase, catalase, and superoxide dismutase were measured²¹⁻²³.

Enzymes of cholesterol metabolism: HMG-CoA reductase in liver was assayed by measuring the formation of coenzyme-A as described by Hulcher and Oleson²⁴. Hepatic cholesterol-7 α -hydroxylase was assayed as described by Petrack and Latario²⁵ using HPLC system (Shimadzu - Model LC 10AVP, Shimadzu Corporation, Kyoto, Japan).

Statistical analysis: Statistical analysis was done using *INSTAT* (version 3.06) statistical software (La Jolla, CA, USA). Results were analyzed by one-way ANOVA and the significance level was calculated using Tukey-Kramer multiple comparison test.

Results

Tender cluster bean pods contained 2.43 g soluble dietary fibre/100 g and a total dietary fibre content of 7.6 g per cent. In terms of cluster bean powder included in the diets, 10 per cent freeze-dried powder of tender cluster beans corresponded to 1.22 per cent soluble dietary fibre and about 3.8 per cent total dietary fibre.

Efficacy of the combination of *C. tetragonoloba* and garlic on CGS formation during experimental induction: To assess the additive beneficial effect of tender cluster beans (CB) and garlic (G) on experimental induction of CGS, test diets incorporating 10 per cent CB, one per cent garlic and 10 per cent CB + 1 per cent garlic in the lithogenic diet were used. Fig.1 summarizes the reduction in CGS formation by these three dietary interventions, which were to an extent of 44.4, 25.1 and 56.3 per cent in mice fed with CB, garlic, CB+garlic, respectively.

Beneficial influence of the combination of *C. tetragonoloba* and garlic on biliary lipid profile during experimental induction of CGS: Biliary cholesterol concentration was increased by 1.8-fold in lithogenic diet fed mice compared to basal control diet fed mice. The same was ameliorated by 34.3, 23.1 and 47.8 per cent upon feeding CB, garlic, and CB+garlic, respectively. Phospholipid content of bile was unaffected in HCD fed group compared to control, and was enhanced by 20 per cent upon feeding CB+garlic. Total bile acid content of bile was increased by 62 per cent in HCD group compared to control and the same was countered by 23, 18, and 30 per cent in animals fed with CB, garlic, and CB+garlic, respectively. The calculated cholesterol saturation index (CSI) was also increased by 2.82 fold in HCD group and the same was ameliorated by 44, 44, and 57 per cent in mice fed with CB, garlic and CB+garlic, respectively (Table I).

Beneficial influence of the combination of *C. tetragonoloba* and garlic on serum lipid profile during experimental induction of CGS: Serum HDL-cholesterol was decreased as a result of HCD feeding (by 24% compared to normal control value) and the same was restored by 18 and 19 per cent, respectively, in mice fed with CB and CB+garlic. LDL-cholesterol was increased in HCD fed animals by 98 per cent and the

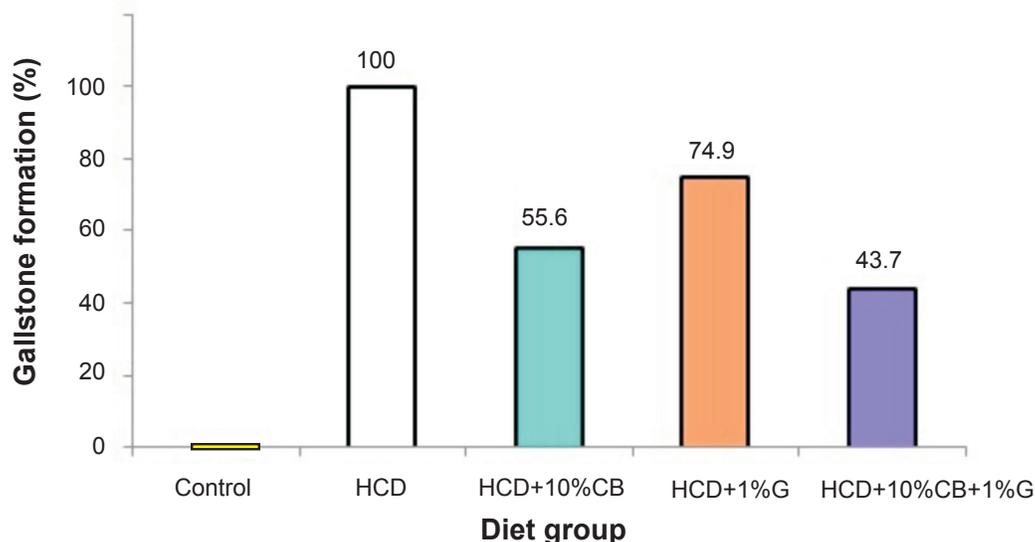


Fig.1. Effect of dietary cluster beans (CB) and garlic (G) on formation of cholesterol gallstone in mice. HCD, high cholesterol diet. Values are mean of 20 animals in each group.

Table I. Effect of dietary cluster beans (CB) and garlic on biliary lipid profile in high cholesterol diet (HCD) fed mice

Animal group	Total lipid (g/dl)	Bile acid (mmol/l)	Cholesterol (mmol/l)	Phospholipid (mmol/l)	CSI	Cholesterol: bile acid ratio	Cholesterol: phospholipid ratio
Control	7.62 ± 0.28*	96.2 ± 5.53*	11.0 ± 1.05*	33.8 ± 1.01	0.71 ± 0.03*	0.11 ± 0.009*	0.33 ± 0.03*
HCD	11.6 ± 0.46	155.2 ± 8.94	31.2 ± 0.79	32.2 ± 0.99	2.00 ± 0.11	0.27 ± 0.009	1.31 ± 0.04
10% CB	9.58 ± 0.62*	119.4 ± 6.26*	20.2 ± 1.80*	32.0 ± 1.15	1.12 ± 0.08*	0.17 ± 0.007*	0.63 ± 0.05*
1% garlic	9.71 ± 0.50*	127.2 ± 8.11*	24.0 ± 1.03*	34.5 ± 3.08	1.13 ± 0.05*	0.18 ± 0.007*	0.70 ± 0.03*
10% CB + 1% garlic	9.09 ± 0.46*	109.2 ± 9.28*	16.3 ± 0.94*	40.2 ± 3.81*	0.86 ± 0.06*	0.15 ± 0.003*	0.41 ± 0.04*

CSI, Cholesterol saturation index
 Values are mean ± SEM of five samples each being pooled from four animals
 *P<0.05 compared with HCD group

same was reversed by 23, 20 and 35 per cent in animals fed with CB, garlic and CB+garlic. Total cholesterol level in test diets CB, garlic and CB+garlic fed animals were reduced by 16, 17 and 24 per cent whereas in the case of HCD fed mice the same was higher by 50 per cent compared to basal control. Phospholipid content of serum was decreased by 26 per cent in HCD fed mice, whereas the same was increased by 15, 25 and 39 per cent in CB, garlic and CB+garlic fed animals. Triglyceride was about 89 per cent higher in HCD group as compared to control value and in test diets CB, garlic and CB+garlic fed animals the same was decreased by 21, 15 and 24 per cent in serum. Increase

in cholesterol to phospholipid ratio caused by HCD was significantly countered by dietary CB, garlic, and their combination (Table II).

Beneficial influence of the combination of C. tetragonoloba and garlic on liver lipid profile during experimental induction of CGS: Liver total lipids increased by 105 per cent in HCD fed animals whereas the same was decreased by 27.5, 12.6, and 30 per cent respectively in CB, garlic and CB+garlic fed mice. Cholesterol was increased as a result of HCD which was 3.5-fold of the normal control value and the same was reduced by 54, 24 and 58 per cent, respectively

in CB, garlic and CB+garlic fed mice. Phospholipid content of liver was decreased by 23 per cent in HCD group, whereas the same was restored by 33, 28.5 and 47 per cent in dietary interventions with CB, garlic, and CB+garlic. Cholesterol: phospholipid ratio was increased in HCD group and the same was reversed in dietary CB, garlic and CB+garlic fed animals (Table III).

HMG-CoA reductase activity in liver was decreased in HCD group because of external cholesterol provided in the diet, whereas the same was increased significantly in CB, garlic and CB+garlic fed mice compared to HCD group (Fig. 2A). Activity of hepatic cholesterol-7 α -hydroxylase on the other hand, was significantly elevated in HCD group (Fig. 2B), and the same was decreased significantly in CB, garlic and CB+garlic fed mice compared to HCD group (Fig. 2B).

As shown in Table IV, liver weight was increased in HCD fed animals by 63 per cent while the same was reversed by 24, 13.8 and 37.5 per cent in CB, garlic and CB+garlic groups. Gallbladder weight was

also increased because of HCD, and this increase was countered by feeding the three test diets, the combination producing the maximum effect. Body weight of the animals remained largely unaffected in the HCD and other dietary interventions.

Beneficial influence of the combination of C. tetragonoloba and garlic on the antioxidant status of serum and liver during experimental induction of CGS: Ascorbic acid and glutathione were significantly decreased in HCD fed animals. In the case of animals fed with test diets CB, garlic and CB+garlic, concentration of these two antioxidant molecules were restored. Lipid peroxide value was high in HCD feeding, and the same was reduced by 54, 27 and 57 per cent in CB, garlic, and CB+garlic fed animals, respectively. Activities of antioxidant enzymes (glutathione peroxidase, glutathione reductase, glutathione-S-transferase, catalase, and superoxide dismutase) were significantly increased in HCD fed animals. Activities of superoxide dismutase and glutathione-S-transferase were further increased in animals fed with CB+garlic in comparison with HCD group (Table V).

Table II. Effect of dietary cluster beans (CB) and garlic on serum lipid profile in high cholesterol diet (HCD) fed mice

Animal group	Total cholesterol (mg/dl)	HDL-cholesterol (mg/dl)	LDL-cholesterol (mg/dl)	Phospholipid (mg/dl)	Triglycerides (mg/dl)	Cholesterol: phospholipid ratio
Control	112.5 \pm 6.24*	44.8 \pm 2.65*	67.5 \pm 3.91*	226.2 \pm 12.94*	112.8 \pm 9.30*	0.497 \pm 0.028*
HCD	169.3 \pm 9.07	34.2 \pm 1.21	133.9 \pm 9.63	167.2 \pm 7.85	213.5 \pm 14.2	1.012 \pm 0.054
10% CB	142.9 \pm 7.68*	40.2 \pm 2.18*	102.9 \pm 5.97*	192.0 \pm 8.22*	168.9 \pm 10.2*	0.744 \pm 0.040*
1% garlic	140.1 \pm 5.78*	32.8 \pm 3.62	107.2 \pm 6.61*	208.8 \pm 10.3*	180.5 \pm 11.5*	0.671 \pm 0.028*
10% CB + 1% garlic	128.3 \pm 5.76*	40.8 \pm 2.77*	87.3 \pm 6.95*	233.1 \pm 14.3*	161.3 \pm 10.8*	0.550 \pm 0.025*

Values are mean \pm SEM of five samples each being pooled from four animals
*P<0.05 compared with HCD group

Table III. Effect of dietary cluster beans (CB) and garlic on liver lipid profile in high cholesterol diet (HCD) fed mice

Animal group	Total lipid (mg/g)	Cholesterol (mg/g)	Phospholipid (mg/g)	Triglycerides (mg/g)	Cholesterol: phospholipid ratio
Control	95.6 \pm 4.10*	16.5 \pm 1.13*	63.0 \pm 4.33*	39.5 \pm 2.66*	0.26 \pm 0.018*
HCD	198.2 \pm 8.20	57.6 \pm 5.42	39.7 \pm 2.69	57.9 \pm 5.98	1.45 \pm 0.140
10% CB	143.6 \pm 5.07*	26.3 \pm 1.75*	52.8 \pm 4.21*	44.6 \pm 2.53*	0.50 \pm 0.033*
1% garlic	173.2 \pm 6.13*	43.5 \pm 3.94*	51.0 \pm 3.01*	52.4 \pm 4.19*	0.85 \pm 0.077*
10% CB + 1% garlic	140.5 \pm 7.86*	24.3 \pm 2.18*	58.2 \pm 4.75*	42.3 \pm 2.71*	0.42 \pm 0.038*

Values are mean \pm SEM of five samples each being pooled from four animals
*P<0.05 compared with HCD group

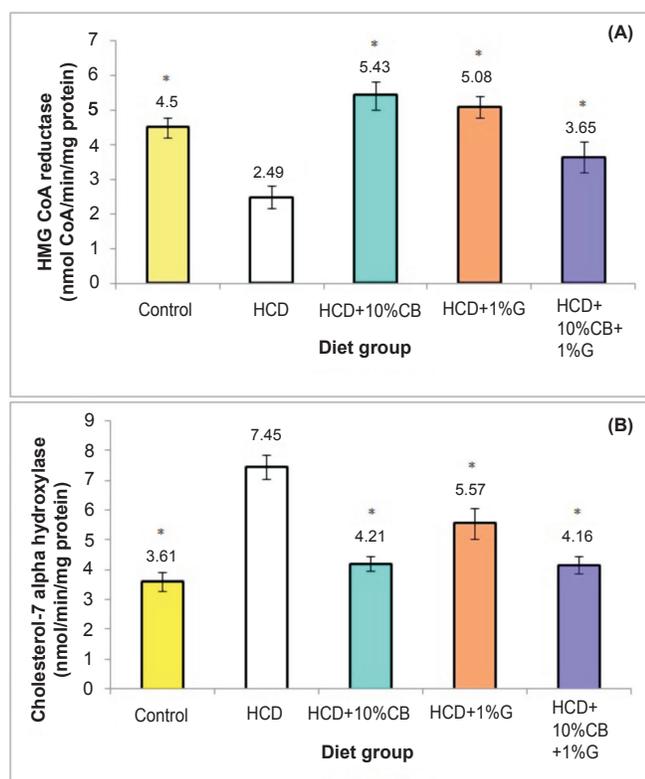


Fig. 2. Effect of dietary cluster beans (CB) and garlic (G) on liver (A) HMG-CoA reductase activity and (B) cholesterol-7 α -hydroxylase activity. HCD, high cholesterol diet. Values are mean \pm SEM of five samples each being pooled from four animals. * P <0.05 compared with HCD group.

There was a significant increase in the activities of all the antioxidant enzymes, *viz.*, glutathione peroxidase, glutathione reductase, glutathione-S-transferase, catalase and superoxide dismutase in HCD fed animals compared to basal control group. The elevated enzyme activities were countered to a large extent in animals fed with CB, garlic and CB+garlic. Serum lipid peroxide concentrations which were

elevated in HCD group were alleviated by the three dietary interventions, the combination of CB and garlic producing the maximum effect (Table VI).

Discussion

Soluble dietary fibre such as guar gum from *C. tetragonoloba* has been proven to be effective in lowering circulatory and hepatic cholesterol by reducing the absorption of dietary cholesterol⁶. The present study was carried out to evaluate the possible additive effect of CB and garlic during induction of cholesterol gallstone in lithogenic diet fed mice. The cholesterol gallstone formation was reduced by the combination of CB and garlic as compared to these two individual ingredients, although it did not amount to an additive effect. Cholesterol secretion into bile was also decreased in CB and garlic fed animals with an increase in phospholipid content compared to high cholesterol diet fed mice.

The anti-lithogenic effect of fibre is partly mediated by a lowered absorption of bile acids in the intestine due to the interruption of the enterohepatic circulation of the same, thus increasing faecal excretion of bile acid, and increased conversion of cholesterol to bile acids in the hepatic tissue²⁶. Additionally, fermentation of dietary fibre by the intestinal microbiota could modify the production of short chain fatty acids especially decreasing acetate production and increasing the propionate synthesis. This in turn reduces the endogenous synthesis of cholesterol²⁶.

The present study indicated that the addition of CB, garlic or CB+garlic to HCD had a significant cholesterol lowering effect. In particular, these dietary interventions reduced serum concentration of LDL-cholesterol, which is primarily responsible for reduced cholesterol: phospholipid ratio in serum. Serum HDL-cholesterol which was decreased as a result of

Table IV. Effect of dietary tender cluster beans (CB) and garlic on liver and gallbladder weights in high cholesterol diet (HCD) fed mice

Animal group	Liver weight (g/100 g body weight)	Gallbladder weight (mg)
Control	3.80 \pm 0.20*	13.1 \pm 0.42*
HCD	6.19 \pm 0.29	27.3 \pm 0.92
10% CB	4.68 \pm 0.11*	22.6 \pm 0.32*
1% garlic	5.33 \pm 0.15*	18.8 \pm 1.05*
10% CB + 1% garlic	3.87 \pm 0.05*	15.0 \pm 0.74*

Values are mean \pm SEM of 20 animals in each group

* P <0.05 compared with HCD group

Table V. Effects of dietary tender cluster beans (CB) and garlic on antioxidant status in the liver of high cholesterol diet (HCD) fed mice

Animal group	Lipid peroxides ($\mu\text{mol MDA}/\text{mg protein}$)	Ascorbic acid ($\mu\text{g}/\text{mg protein}$)	Glutathione ($\text{nmol}/\text{mg protein}$)	Glutathione peroxidase ($\mu\text{mol}/\text{min}/\text{mg protein}$)	Glutathione reductase ($\mu\text{mol}/\text{min}/\text{mg protein}$)	Glutathione-S- transferase ($\mu\text{mol}/\text{min}/\text{mg protein}$)	Catalase ($\mu\text{mol}/\text{min}/\text{mg protein}$)	Superoxide dismutase (Units/min/mg protein)
Control	3.48 \pm 0.26*	80.6 \pm 5.02*	37.8 \pm 1.70*	22.6 \pm 1.90*	88.9 \pm 2.68*	47.9 \pm 3.21*	48.1 \pm 3.06*	26.1 \pm 1.46*
HCD	16.4 \pm 0.96	54.1 \pm 3.59	22.4 \pm 1.89	37.6 \pm 2.14	138.4 \pm 6.15	68.0 \pm 5.36	78.9 \pm 4.38	31.0 \pm 1.55
10 % CB	7.51 \pm 0.90*	72.0 \pm 3.47*	30.0 \pm 1.97*	32.1 \pm 2.28*	104.8 \pm 5.73*	67.8 \pm 6.20	67.9 \pm 3.45*	40.8 \pm 3.26*
1% garlic	12.0 \pm 0.89*	69.3 \pm 3.01*	31.2 \pm 1.72*	29.1 \pm 1.64*	99.1 \pm 5.97*	85.0 \pm 1.08*	70.0 \pm 5.27	38.8 \pm 2.08*
10% CB + 1% garlic	7.05 \pm 0.30*	73.3 \pm 3.75*	36.9 \pm 2.67*	28.9 \pm 0.83*	102.9 \pm 5.80*	101.6 \pm 3.97*	46.3 \pm 2.35*	43.8 \pm 2.23*

Values are mean \pm SEM of five samples each being pooled from four animals
* $P < 0.05$ compared with HCD group

lithogenic diet was countered in mice fed with CB, garlic and their combination. The combination of CB and garlic also produced higher beneficial effect than the individual CB or garlic on serum cholesterol and phospholipids and hence cholesterol: phospholipid ratio.

The significant decrease in liver weight with the incorporation of CB or garlic into HCD was a result of lowered lipid concentration in the hepatic tissue. The lowering of cholesterol: phospholipid ratio in liver was a result of reduction in hepatic cholesterol and triglyceride, and an increase in hepatic phospholipids. Decreased hepatic cholesterol by the dietary interventions could be due to multiple effects on hepatic cholesterol homeostasis *viz.* downregulation of HMG-CoA reductase activity and interfering with dietary cholesterol absorption. The activity of hepatic HMG-CoA reductase decreased in HCD fed animals, and was reversed in CB and garlic fed animals, thus countering the effect of HCD.

Diets rich in cholesterol produce lithogenic bile and gallstones in experimental mice³. Continued intake of HCD increased cholesterol secretion and hence elevated the cholesterol: phospholipid ratio, cholesterol: bile acid ratio and cholesterol saturation index in the bile, which subsequently resulted in cholesterol crystallization. Supplementation of CB, garlic or CB+garlic resisted these changes thus inhibiting cholesterol crystallization in gallbladder. A positive relation between LDL-cholesterol and CGS²⁷ and an inverse relation between plasma total HDL or HDL₃ cholesterol and gallstone formation²⁸ have been reported and hence a protective role of HDL cholesterol against CGS is suggested. We have earlier shown that dietary CB reduces serum LDL-cholesterol and the hypocholesterolaemic effect of tender cluster beans is attributable to the increased faecal excretion of bile acids and neutral steroids²⁹.

Thus, incorporation of garlic along with tender cluster beans produced a greater reduction in the formation of CGS caused by HCD. Tender cluster beans and garlic either individually or together moderated the cholesterol saturation index in bile. The anti-cholelithogenic influence was associated with a reduction in serum LDL-cholesterol. Interventions with CB and garlic individually or in combination significantly reduced fat accumulation in liver. In addition to anti-lithogenic influence, dietary CB, garlic or their combination had a beneficial antioxidant influence under lithogenic condition. Although there

Table VI. Effect of dietary cluster beans (CB) and garlic on antioxidant status in the serum of high cholesterol diet (HCD) fed mice

Animal group	Lipid peroxides ($\mu\text{mol MDA/mg}$ protein)	Glutathione peroxidase ($\mu\text{mol/min/mg}$ protein)	Glutathione reductase ($\mu\text{mol/min/mg}$ protein)	Glutathione-S- transferase ($\mu\text{mol/min/mg}$ protein)	Catalase ($\mu\text{mol/min/mg}$ protein)	Superoxide dismutase (Units/min/mg protein)
Control	5.02 \pm 0.20*	9.23 \pm 0.32*	3.30 \pm 0.30*	4.91 \pm 0.27*	0.32 \pm 0.02*	2.41 \pm 0.16*
HCD	15.0 \pm 0.76	12.1 \pm 0.94	3.99 \pm 0.13	10.8 \pm 0.76	0.45 \pm 0.05	3.67 \pm 0.25
10% CB	9.33 \pm 0.31*	9.86 \pm 0.35*	3.72 \pm 0.17	6.87 \pm 0.35*	0.32 \pm 0.03*	2.55 \pm 0.07*
1% garlic	11.5 \pm 0.51*	9.69 \pm 0.46*	3.48 \pm 0.22	7.17 \pm 0.54*	0.39 \pm 0.04	2.94 \pm 0.11*
10% CB + 1% garlic	7.56 \pm 0.41*	8.71 \pm 0.26*	3.16 \pm 0.19*	6.84 \pm 0.34*	0.28 \pm 0.04*	2.47 \pm 0.08*

Values are mean \pm SEM of five samples each being pooled from four animals

* $P < 0.05$ compared with HCD group

was no additive effect with respect to the modulation of lipid homeostasis or antioxidant molecules and the activity of antioxidant enzymes, the beneficial effect was higher with the combination of CB and garlic as compared to the individual effect of the components. The anti-cholelithogenic influence of dietary CB remains to be understood in conditions of pre-established CGS.

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Conflicts of Interest: None.

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