JOURNAL OF
AGRICULTURAL AND
FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Potentiation of Hypolipidemic and Weight-Reducing Influence of Dietary Tender Cluster Bean (*Cyamopsis tetragonoloba*) When Combined with Capsaicin in High-Fat-Fed Rats

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ABSTRACT: Soluble fiber-rich tender cluster bean pod (*Cyamopsis tetragonoloba*) (CB) and a combination of CB and capsaicin, a thermogenic spice compound, were evaluated for weight-reducing effect in high-fat-fed Wistar rats. Freeze-dried CB and capsaicin were included at 15 and 0.015%, respectively, in the high-fat (30%) diet for 8 weeks. Excretion of dietary fat, fat deposition in adipose, and activities of enzymes involved in lipolysis and lipogenesis were examined. CB and capsaicin additively decreased weight gain in high-fat-fed rats, without affecting feed intake. The antiobesity potential of CB was through impeding fat accumulation in adipose and enhancing fat excretion. Decrease in adipose triglyceride by the combination was higher than their individual effects and was accompanied by enhanced activity of hormone-sensitive lipase-facilitating mobilization of depot fat. Increased fecal excretion of triglycerides by the combination of CB and capsaicin was additive. Whereas the hypolipidemic effect of the combination was higher than their individual effects, the decrease in hepatic cholesterol and triglycerides produced by the combination was additve. Thus, dietary CB significantly checked weight gain and adverse changes in lipid profile in high-fat-fed condition amounting to a cardioprotective effect. These beneficial effects were potentiated by coadministration of capsaicin.

KEYWORDS: *capsaicin, high-fat diet, fat absorption, tender cluster beans, weight management*

INTRODUCTION

Excess body weight is an important risk factor for morbidity and mortality,[1] with increased risk of type 2 diabetes, osteoarthritis of weight-bearing joints, cardiovascular disease, certain cancers, and musculoskeletal disorders.[2] The prevalence of obesity and overweight is increasing in developed countries due to change in lifestyle. Most researchers believe that overweight and obesity are caused by numerous factors; however, a long-term energy imbalance between intake and expenditure appears to be the primary cause. Hence, limiting energy consumption may be an important strategy for weight control. Over the years, much attention has been devoted to prescribing the optimal amount of food energy to achieve a healthy body weight. Some researchers have also investigated the possibility of consuming specific dietary components that would aid in weight management. Fiber consumption is a dietary approach that has received substantial attention in this context. Experimental evidence on dietary fiber being inversely related to the weight gain and also epidemiological evidence have suggested a strong inverse association between intake of dietary fiber and obesity and the risk of coronary heart disease.[3] Moreover, vegetarian populations have a lower prevalence of obesity, which suggests that their high fiber intake associated with plant-derived foods could play an important role in the prevention of obesity.[4]

Dietary fiber intake may modulate parameters associated with the control of metabolic syndrome, namely, food intake through an effect on satiety, and consequently modulate glucose homeostasis, insulin sensitivity, glycemia, insulinemia, and blood lipids.[5] The fermentable dietary fiber seems to be important to produce specific effects on satiety and glycemia through the release of gut peptides. Guar gum is a naturally occurring soluble dietary fiber present in cluster bean, which is chemically a galactomannan polysaccharide.[6] This fiber undergoes degradation in the large intestine by the microbial enzymes, producing short-chain fatty acids that are beneficial to human health.[6]

In the context of lower incidence of obesity in vegetarian populations and the existing knowledge that soluble dietary fiber may have an inverse relationship with weight gain, the present animal study investigated a possible beneficial role of dietary tender cluster beans as a source of soluble dietary fiber in weight management in high-fat-fed rats. In this context, gain in body weight, fecal excretion of dietary fat, fat absorption and deposition in adipose tissue, and activities of enzymes involved in liposlysis and lipogenesis were examined. The hypolipidemic and antiobesity actions of dietary red pepper (*Capsicum annuum*) and its pungent principle, capsaicin, are known.[7] The mode of action of capsaicin in exerting the hypolipidemic and weight-reducing effects is different from that of dietary soluble fiber. Hence, there is a possibility of an additive effect or even a synergistic effect when this spice is consumed in combination with dietary soluble fiber. Because capsaicin promotes fat oxidation in the body,[7] the same was also evaluated in this study for a possible synergistic effect when fed along with tender cluster beans.

MATERIALS AND METHODS

Chemicals. Capsaicin was obtained from Fluka Chemie (Buchs, Switzerland). Corn starch, sugar powder, and refined peanut oil were purchased from a local market. Salt mixture (Bernhardt-Tommarelli

Received: March 19, 2012
Revised: July 14, 2012
Accepted: July 27, 2012
Published: July 27, 2012

dx.doi.org/10.1021/jf301211c | *J. Agric. Food Chem.* 2012, 60, 8155−8162

modified) was from SISCO Research Laboratories (Mumbai, India). 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), dithiothreitol (DTT), malic acid, nicotinamide adenine dinucleotide (NAD), polyvinyl pyrrolidone (PVP-40), coenzyme A, malate dehydrogenase, glucose-6-phosphate, nicotinamide adenine dinucleotide phosphate (NADP), 6-phosphogluconate, reduced nicotinamide adenine dinucleotide (NADH), lactate dehydrogenase, adenosine triphosphate (ATP), glycyl-glycine, heparin, and bovine serum album were procured from Sigma Chemical Co. (St. Louis, MO, USA). Triethanolamine, vitamin E acetate, vitamin A acetate, cholecalciferol, and ethylenediaminetetraacetic acid (EDTA) were obtained from Himedia Laboratories (Mumbai, India). Food grade casein was purchased from Nimesh Corp. (Mumbai, India). Other chemicals and solvents were of analytical grade.

Tender cluster beans were procured from the local supermarket, freeze-dried, and milled to a fine powder. The powdered dry samples were analyzed for dietary fiber as soluble dietary fiber and insoluble dietary fiber by adapting the rapid enzymatic assay procedure of Asp et al.[8]

Animal Treatment. The animal experiment was carried out by taking appropriate measures to minimize pain or discomfort and with due approval from the Institutional Animal Ethics Committee. Male Wistar rats (eight per group) weighing 90−100 g (obtained from the Experimental Animal Production Facility of this Institute) were housed in individual stainless steel cages and maintained on various experimental diets for 8 weeks with free access to food and water. The basal AIN-76 semipurified diet consisted of casein, 21%; cane sugar, 10%; corn starch, 54%; refined peanut oil, 10%; vitaminized starch, 1%; and Bernhardt-Tommarelli modified salt mixture, 4%. The high-fat diet (HFD) consisted of casein, 21%; cane sugar, 10%; corn starch, 34%; hydrogenated fat, 25%; refined peanut oil, 5%; vitaminized starch, 1%; and Bernhardt-Tommarelli modified salt mixture, 4%. Experimental intervention diets consisted of (1) cluster bean powder (15%), (2) capsaicin (0.015%), and (3) cluster bean powder (15%) plus capsaicin (0.015%) incorporated into basal and high-fat diets, replacing an equivalent amount of corn starch. Food consumption was monitored daily, whereas body weight gain was monitored at weekly intervals. Food efficiency ratio was calculated as (body weight change for the experimental period) ÷ (food consumed for the experimental period).

At the end of the experimental regimen, overnight-fasted animals were sacrificed under ether anesthesia. Blood was drawn by cardiac puncture and kept at 4 °C for 3 h. Serum was separated by centrifugation at 1000*g* for 20 min and stored at −20 °C until analysis. Liver was excised and rinsed with ice-cold phosphate-buffered saline (pH 7.4). Whole liver weight was noted, and portions of fresh liver were blotted, weighed, and stored at −20 °C until lipid analysis. Perirenal adipose tissue was excised, weighed, and stored at −20 °C. Triglycerides in adipose were estimated according to the procedure of Fletcher.[9]

Dietary Fat Absorption. Toward the end of the feeding trial, fecal matter was collected for 72 h. Weight of feces was noted, and fecal triglyceride was estimated according to the method of Fletcher.[9] Fat absorption was computed as the difference between fat intake (computed from food intake data) and excretion through feces.

Serum and Liver Lipids. Total lipids in serum and liver were extracted according to the method of Folch et al.[10] Cholesterol in the lipid extract was estimated by using the method of Searcy and Bergquist.[11] Serum cholesterol and triglycerides associated with HDL fraction were determined after precipitation of apolipoprotein-B-containing lipoprotein with heparin−manganese reagent according to the method of Warnick and Albers.[12] LDL−VLDL precipitate was extracted with chloroform/methanol (2:1 v/v) and used for cholesterol determination. Phospholipids were quantitated by ferrous ammonium thiocyante method using dipalmitoylphosphatidylcholine as standard according to the procedure of Stewart.[13] Triglycerides were estimated by using the procedure of Fletcher[9] with tripalmitin as reference standard. Perirenal adipose tissues were homogenized, and lipid was extracted according to the method of Folch et al.;[10] [9]

Activities of Liver Lipogenic Enzymes. Liver was homogenized in cold 0.15 M potassium chloride (10% homogenate) and centrifuged at 10000*g* at 4 °C for 15 min (Sorval RC-2B centrifuge, DuPont Instruments, USA). The resulting postmitochondrial supernatant was used for enzyme assays. Activities of ATP-citrate lyase,[14] glucose-6-phosphate dehydrogenase,[15] 6-phosphogluconate dehydrogenase,[16] and malic enzyme[17] were estimated using standard procedures.

Activity of Hormone-Sensitive Lipase in Adipose Tissue. Hormone-sensitive lipase in perirenal adipose tissue was extracted as described by Khoo and Steinberg.[18] The tissue was homogenized in 4 parts of 0.05 M Tris-HCl (pH 8.2) containing 1 IU heparin/mL and spun at 8000*g* for 30 min. The fat-free clear solution was used for the determination of enzyme activity as described by Belfrage and Vaughan.[19] The enzyme (0.2 mL) was mixed with a buffer−substrate mixture (0.6 mL) containing 40 μmol of phosphate buffer (pH 6.8), 1 μmol of ^{14}C-triolein, 30 μmol of EDTA, and 20 mg of albumin, and the mixture was incubated at 30 °C for 1 h. The reaction was terminated by adding 3 mL of fatty acid extraction mixture (chloroform/methanol/benzene (2:2.4:1 v/v)), containing 0.3 μmol of nonradioactive oleic acid, followed by the addition of 0.1 mL of 1 N sodium hydroxide (final pH 11.0). The mixture was shaken vigorously and centrifuged at 1500*g* for 10 min. An aliquot of the upper phase (1.8 mL) was transferred to a scintillation vial containing 10 mL of scintillation fluid, and radioactivity was measured in a liquid scintillation counter (Perkin-Elmer Tri-Carb 2900TR). The enzyme activity is expressed as micromoles of free fatty acid released per gram of fresh tissue per hour.

Statistical Analysis. Results are expressed as the mean ± SEM, and comparisons between groups were made by means of one-way ANOVA using the Tukey−Kramer multiple-comparison test. Differences were considered to be significant for $p < 0.05$.

RESULTS

Freeze-dried tender cluster bean pods used in this study contained 12.2 g of soluble dietary fiber/100 g and 25.8 g of insoluble dietary fiber/100 g, amounting to a total dietary fiber content of 38% (w/w). In terms of freeze-dried cluster bean powder included in the diets, 15% cluster bean containing diet corresponded to 1.83% soluble dietary fiber, 3.87% insoluble dietary fiber, and 5.7% total dietary fiber.

Influence of Dietary Cluster Beans and Capsaicin on Body Weight and Liver Weight. Weight gain was 33% higher in the HFD group as compared to basal control rats (Figure 1A). CB reduced the weight gain in HFD fed rats by 13% ($p < 0.05$), whereas capsaicin produced a 15% decrease ($p < 0.05$). The two test materials in combination produced a 28% decrease in the body weight gain, amounting to an additive effect ($p < 0.05$). Although CB and capsaicin individually showed a tendency to resist weight gain in the basal diet fed animals (not statistically significant), their combination significantly decreased the weight gain in the animals fed basal diet ($p < 0.05$). Feed intake data (not shown) suggested that CB or capsaicin in the diet did not affect the feed intake. Liver weight was decreased by dietary CB and capsaicin, both individually and in combination (Figure 1B). HFD feeding produced a 65% increase in liver weight. This increase in absolute liver weight was countered by dietary CB and capsaicin by 37 and 29%, respectively, whereas their combination countered the same by 40%, bringing the value closer to that of the basal control group.

Influence of Dietary Cluster Bean and Capsaicin on Adipose Weight and Adipose Triglycerides. There was about a 40% increase in the adipose weight as a result of feeding HFD (Table 1). Weight of adipose tissue was decreased in basal diet as well as in HFD-fed animals by inclusion of CB in the diet. In the HFD-fed situation, dietary CB decreased the

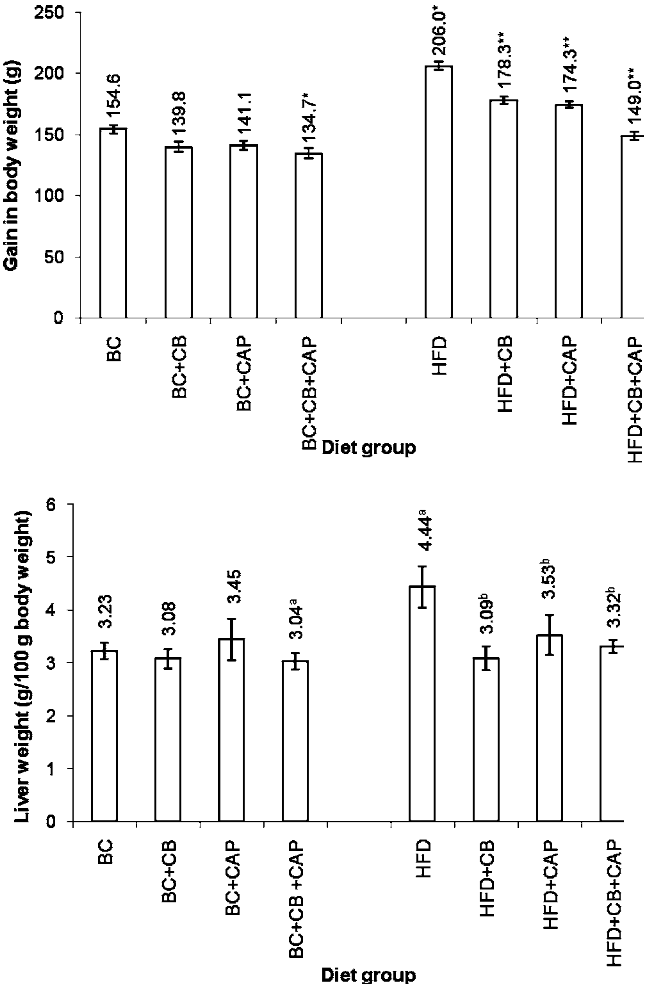

Figure 1. Influence of dietary cluster bean and capsaicin on (A, top) body weight gain and (B, bottom) liver weight of rats fed a high-fat diet. BC, basal control; HFD, high-fat diet; CB, cluster bean; CAP, capsaicin. Values are the mean ± SEM of eight animals per group. [a], significant compared to basal control ($p < 0.05$); [b], significant compared to HFD control ($p < 0.05$).

weight of perirenal adipose tissue by 45.6%, whereas capsaicin and the two in combination lowered it by 40.2 and 48%, respectively. Decreases of 42.3, 40.9, and 52.5% in the weight of adipose tissue were seen upon inclusion of CB, capsaicin, and their combination, respectively, in the basal diet fed condition. Adipose triglyceride content was increased by 54% as a consequence of HFD (Table 1). The reductions in adipose triglyceride content brought about by inclusion of CB and capsaicin along with HFD were about 60 and 56.5%, respectively. The two in combination reduced it by 66%, an effect greater than those of the individual components. In basal groups, CB and capsaicin independently reduced adipose triglyceride content by 53%, whereas the two fed in combination produced a 68% decrease in the same. Thus, the effect of the combination on adipose triglyceride deposition was greater than either of the individual effects.

Influence of Dietary Cluster Beans and Capsaicin on Hormone-Sensitive Lipase. HFD significantly increased the activity of hormone-sensitive lipase in adipose tissue, which was 77.4% higher than the basal control (Table 1). Feeding CB or capsaicin to rats maintained on HFD further increased the activity of this enzyme by 33 and 26%, respectively. CB and capsaicin together produced a 63% increase in the enzyme activity. Thus, the combination of CB and capsaicin along with HFD produced an additive effect in increasing the activity of hormone-sensitive lipase in adipose tissue, which facilitates mobilization of adipose fat by these dietary interventions in a high-fat-fed state. The same trend was seen in basal diet groups, where feeding CB and capsaicin increased the activity of this enzyme by 85 and 90%, whereas the combination of CB and capsaicin increased the enzyme activity by 119%.

Influence of Dietary Cluster Beans and Capsaicin on Serum Lipid Profile. Dietary CB and capsaicin showed significant hypolipidemic effect both independently and in combination in hypertriglyceridemic rats (Table 2). Feeding HFD resulted in about a 14% increase in circulating total cholesterol. A 17.2% decrease in circulating cholesterol was seen in the group of rats fed HFD+CB ($p < 0.5$). The HFD+CAP group showed an 18.6% reduction in circulating cholesterol, whereas the HFD+CB+CAP group showed a 20.8% reduction in the same. Incidentally, a significant reduction in serum cholesterol was also seen in basal groups with these two dietary interventions; 12% decreases were observed by feeding CB and capsaicin individually, whereas the combination of the two produced a decrease of 20.2%. The decrease in serum cholesterol brought about by dietary CB and capsaicin was seen particularly in the LDL–VLDL fraction; the lowering of circulating LDL–VLDL associated cholesterol by CB and capsaicin was not additive. The HFD elevated serum

Table 1. Effect of Dietary Cluster Bean and Capsaicin on Adipose Tissue Weight, Triglycerides, and Activity of Hormone-Sensitive Lipase in High-Fat-Fed Rats[a]

	adipose weight		adipose triglyceride		
diet group[b]	g	g/100 g body weight	mg/g tissue	mg/100 g body weight	hormone-sensitive lipase in adipose tissue (mol FFA released/h/mg tissue)
BC	2.72 ± 0.03	1.098 ± 0.012	901.3 ± 20.2	989.6 ± 22.2	1.594 ± 0.125
BC+CB	1.47 ± 0.03 a	0.634 ± 0.012 a	728.9 ± 20.0 a	462.1 ± 12.7 a	2.960 ± 0.161 a
BC+CAP	1.53 ± 0.05 a	0.649 ± 0.021 a	709.9 ± 26.6 a	460.9 ± 17.3 a	2.875 ± 0.209 a
BC+CB+CAP	1.20 ± 0.10 a	0.522 ± 0.32 a	606.3 ± 19.1 a	316.5 ± 10.0 a	3.503 ± 0.254 a
HFD	4.19 ± 0.34 a	1.54 ± 0.124 a	989.5 ± 20.7 a	1523.8 ± 31.9 a	2.828 ± 0.229 a
HFD+CB	2.10 ± 0.06 b	0.837 ± 0.023 b	733.8 ± 24.8 b	614.2 ± 20.7 b	3.761 ± 0.312 b
HFD+CAP	2.45 ± 0.06 b	0.921 ± 0.022 b	719.6 ± 31.4 b	662.8 ± 28.9 b	3.544 ± 0.318 b
HFD+CB +CAP	2.02 ± 0.04 b	0.800 ± 0.015 b	644.2 ± 29.8 b	515.4 ± 23.8 b	4.610 ± 0.416 b

[a]Values expressed are the mean ± SEM of eight animals per group: a, significant compared to basal control ($p < 0.05$); b, significant compared to HFD control ($p < 0.05$). [b]BC, basal control; HFD, high-fat diet; CB, cluster bean; CAP, capsaicin.

Table 2. Influence of Dietary Cluster Beans and Capsaicin on Serum Lipid Profile in High-Fat-Fed Rats[a]

	cholesterol						
diet group[b]	total	LDL−VLDL	HDL	triglyceride	phospholipid	cholesterol/ phospholipid ratio	atherogenicity index
BC	77.3 ± 3.23	38.6 ± 1.53	34.9 ± 1.76	146.0 ± 7.71	75.6 ± 2.25	1.022 ± 0.055	1.106 ± 0.043
BC+CB	68.0 ± 2.59 a	25.8 ± 2.43 a	38.8 ± 1.01	116.8 ± 6.20	76.1 ± 3.98	0.894 ± 0.035 a	0.664 ± 0.063 a
BC+CAP	68.1 ± 4.17 a	26.9 ± 0.63 a	37.0 ± 1.07	128.3 ± 6.52	79.7 ± 1.59	0.855 ± 0.052 a	0.727 ± 0.037 a
BC+CB+CAP	61.7 ± 1.38 a	23.9 ± 1.49 a	37.5 ± 1.13	118.7 ± 7.60	74.8 ± 2.61	0.825 ± 0.028 a	0.637 ± 0.039 a
HFD	87.8 ± 2.24 a	47.5 ± 1.67 a	34.2 ± 1.98	241.6 ± 9.51 a	74.0 ± 2.60 a	1.186 ± 0.050 a	1.388 ± 0.049 a
HFD+CB	72.7 ± 4.43 b	30.6 ± 1.80 b	37.9 ± 1.79	122.6 ± 10.1 b	80.9 ± 2.43 b	0.626 ± 0.054 b	0.807 ± 0.047 b
HFD+CAP	71.5 ± 3.90 b	33.8 ± 1.43 b	32.7 ± 3.08	122.3 ± 7.59 b	84.8 ± 2.61 b	0.619 ± 0.046 b	1.033 ± 0.043 b
HFD+CB+CAP	69.5 ± 1.64 b	29.3 ± 1.85 b	37.5 ± 2.69	91.4 ± 8.39 b	91.6 ± 2.50 b	0.540 ± 0.020 b	0.781 ± 0.043 b

[a] Values expressed (mg/dL) are the mean ± SEM of eight animals per group: a, significant compared to basal control ($p < 0.05$); b, significant compared to HFD control ($p < 0.05$). [b] BC, basal control; HFD, high-fat diet; CB, cluster bean; CAP, capsaicin.

Table 3. Effect of Dietary Cluster Bean and Capsaicin on the Liver Lipid Profile of Rats Fed a High-Fat Diet[a]

diet group[b]	cholesterol	triglycerides	phospholipids	cholesterol/phospholipid ratio
BC	9.79 ± 0.311	12.4 ± 0.25	24.7 ± 0.80	0.396 ± 0.013
BC+CB	7.54 ± 0.132 a	10.7 ± 0.93 a	26.7 ± 1.23	0.282 ± 0.005 a
BC+CAP	8.96 ± 0.260 a	11.1 ± 0.57 a	27.6 ± 0.91	0.325 ± 0.010
BC+CB+CAP	6.38 ± 0.313 a	8.32 ± 1.28 a	28.8 ± 1.14	0.221 ± 0.010 a
HFD	12.8 ± 0.852 a	29.8 ± 2.21 a	25.1 ± 0.75	0.509 ± 0.036 a
HFD+BC	10.7 ± 0.641 b	13.7 ± 1.21 b	31.2 ± 1.56 b	0.341 ± 0.020 b
HFD+CAP	11.5 ± 0.180	16.0 ± 0.77 b	27.4 ± 1.23	0. 418 ± 0.016 b
HFD+CB+CAP	9.04 ± 0.182 b	8.95 ± 1.35 b	30.4 ± 0.98 b	0.297 ± 0.008 b

[a] Values expressed as mg/g fresh tissue are the mean ± SEM of eight animals per group: a, significant compared to basal control ($p < 0.05$); b, significant compared to HFD control ($p < 0.05$). [b] BC, basal control; HFD, high-fat diet; CB, cluster bean; CAP, capsaicin.

triglyceride levels by 65.2%, whereas inclusion of CB and capsaicin in the HFD significantly countered this elevation (Table 2). Inclusion of either CB or capsaicin in the HFD brought a decrease of about 49% in serum triglycerides, whereas the combination of the two decreased it by 62.2%, which is greater than the individual effects. Dietary CB and capsaicin showed a hypotriglyceridemic effect even in the basal diet groups. Whereas CB produced a 20% decrease and capsaicin showed a 12% decrease, the combination of the two showed a 20% decrease in circulating triglycerides. Although HFD did not affect the circulating phospholipids, inclusion of CB and capsaicin independently increased serum phospholipid concentration in high-fat-fed animals, the increases being about 10 and 14.5%, respectively (Table 2). The combination of CB and capsaicin along with HFD increased serum phospholipids by 23.7%.

The atherogenicity index in HFD-fed rats was 25% higher than that of basal control rats, thereby elevating the risk of CVD (Table 2). This index was reduced in the groups of rats fed CB and capsaicin in HFD as well as in basal groups ($p < 0.5$). The increase in atherogenicity index was countered by dietary CB and capsaicin by 42 and 26%, respectively, whereas the combination of the two lowered the same by 44% in the HFD-fed situation. The same trend was seen in basal diet fed rats, where dietary CB lowered the atherogenicity index by 40%, but capsaicin and the combination of the two lowered the atherogenicity index by 34 and 42%, respectively. Dietary intervention by CB and capsaicin reduced the HFD-induced elevated cholesterol/phospholipid ratio in the serum by 47 and 47.5%, respectively, whereas the combination of the two decreased it by 54.2% (Table 2). In the basal diet groups, CB and capsaicin decreased the cholesterol/phospholipid ratio by 12.4 and 16.2%, respectively, whereas their combination brought about a 19% lowering of the same.

Influence of Dietary Cluster Beans and Capsaicin on the Hepatic Lipid Profile. The increase in hepatic cholesterol by HFD was countered to a significant extent individually by dietary CB and capsaicin (16.5 and 10.2% decrease, respectively) (Table 3), with their combination producing a 29.4% decrease, thus amounting to an additive effect of the two ingredients. The same trend was seen in basal diet groups also, where CB produced a 22.9% decrease and capsaicin produced an 8.4% decrease in hepatic cholesterol, whereas their combination produced a 34.8% decrease. HFD feeding resulted in a 140% increase in liver triglycerides, which was effectively countered by inclusion of CB or capsaicin individually (54.2 and 46.2% decrease, respectively) (Table 3). The combination of CB and capsaicin produced a 70% decrease in hepatic triglycerides in the high-fat-fed situation. Thus, the combination of CB and capsaicin lowered triglycerides more than the individual effects of these two ingredients. In basal diet groups, CB and capsaicin lowered hepatic triglyceride by 13.8 and 11.3%, respectively ($p < 0.05$), whereas the combination lowered it by 33%, which amounted to an additive effect of the two ingredients. Hepatic phospholipid was enhanced by CB by 24.3%, whereas the combination of CB and capsaicin elevated the same by 21.1% in the HFD group. As a result of enhanced cholesterol content, high-fat-fed rats showed an elevated cholesterol/phospholipid ratio, which was 28% higher compared to basal control rats (Table 3). Feeding CB decreased this ratio by 33%, whereas capsaicin decreased the ratio by 18% in the high-fat-fed condition. The two in combination lowered it by 42%. In basal diet groups, CB decreased the hepatic cholesterol/phospholipid ratio by 29%, whereas feeding capsaicin resulted in an 18% decrease. The

Table 4. Effect of Dietary Cluster Bean and Capsaicin on Hepatic Lipogenic Enzymes in High-Fat-Fed Rats[a]

diet group[b]	malic enzyme (ΔE/h/mg protein)[c]	ATP-citrate lyase (ΔE/h/mg protein)	6-PGDH[d] (ΔE/h/mg protein)	G-6PDH[e] (ΔE/h/mg protein)
BC	1.578 ± 0.240	3.258 ± 0.121	14.1 ± 0.912	7.380 ± 1.240
BC+CB	1.158 ± 0.121 a	2.372 ± 0.422 a	8.94 ± 1.202 a	3.588 ± 0.660 a
BC+CAP	0.570 ± 0.036 a	1.291 ± 0.120 a	4.62 ± 0.242 a	3.541 ± 0.244 a
BC+CB+CAP	0.606 ± 0.012 a	1.686 ± 0.125 a	4.80 ± 0.240 a	3.420 ± 0.234 a
HFD	0.606 ± 0.036 a	2.064 ± 0.128 a	4.22 ± 0.174 a	2.822 ± 0.183 a
HFD+CB	0.636 ± 0.012	2.412 ± 0.305 b	4.98 ± 0.302 b	3.302 ± 0.298
HFD+CAP	0.654 ± 0.018	2.322 ± 0.183 b	5.16 ± 0.180 b	3.542 ± 0.304 b
HFD+CB+CAP	0.780 ± 0.060 b	2.826 ± 0.122 b	5.88 ± 0.184 b	4.204 ± 0.180 b

[a] Values expressed are the mean ± SEM of eight animals per group: a, significant compared to basal control ($p < 0.05$); b, significant compared to HFD control ($p < 0.05$). [b] BC, basal control; HFD, high-fat diet; CB, cluster bean; CAP, capsaicin. [c] ΔE, change in absorbance. [d] 6-PGDH, 6-phosphogluconate dehydrogenase. [e] G6PDH, glucose-6-phosphate dehydrogenase.

Table 5. Effect of Dietary Cluster Bean and Capsaicin on Dietary Fat Absorption in Rats Fed a High-Fat Diet[a]

diet group[b]	fat intake (g/day)	fecal triglycerides (g/day)	triglyceride absorption	
			g/day	% dietary triglyceride
BC	1.35 ± 0.04	0.072 ± 0.002	1.23 ± 0.03	91.1
BC+CB	1.38 ± 0.05	0.169 ± 0.028 a	1.21 ± 0.03	87.7
BC+CAP	1.37 ± 0.05	0.210 ± 0.019 a	1.16 ± 0.03	84.7
BC+CB+CAP	1.28 ± 0.05	0.280 ± 0.028 a	1.00 ± 0.02 a	78.1
HFD	3.13 ± 0.09 a	0.450 ± 0.037 a	2.68 ± 0.05	85.6
HFD+CB	3.13 ± 0.18	0.650 ± 0.052 b	2.48 ± 0.13	79.2
HFD+CAP	3.02 ± 0.10	0.702 ± 0.034 b	2.32 ± 0.07 b	76.8
HFD+CB+CAP	3.14 ± 0.13	0.853 ± 0.042 b	2.29 ± 0.09 b	72.9

[a] Values expressed are the mean ± SEM of eight animals per group: a, significant as compared to basal control ($p < 0.05$); b, significant when compared to HFD control ($p < 0.05$). [b] BC, basal control; HFD, high-fat diet; CB, cluster bean; CAP, capsaicin.

combination of the two produced a 45% decrease. Thus, in both normal and high-fat-fed states, the two in combination produced an effect that was greater than those of individual components, suggesting an additive effect of CB and capsaicin in reverting the elevated cholesterol/phospholipid ratio in the liver.

Influence of Dietary Cluster Beans and Capsaicin on the Activities of Hepatic Lipogenic Enzymes. Feeding HFD significantly suppressed the activity of hepatic lipogenic enzymes as a part of the body's natural homeostatis (Table 4). The test diets, CB, CAP, or their combination, countered the HFD-induced decrease in the activity of these enzymes, demonstrating a revival of endogenous fat synthesis to some extent by impeding the absorption of dietary fat and accelerating the removal of depot fat. The activity of ATP-citrate lyase in the liver, which provides acetyl CoA for lipogenesis, was decreased by 37% in rats fed HFD. HFD enriched with CB increased its activity by 17%, whereas CAP increased it by 12.5%. The combination of the two present along with HFD caused a 37% increase in its activity, which suggests an additive effect of the individual constituents. In the basal diet groups, CB, CAP, and their combination caused reductions in the activity of this enzyme by 27, 60, and 49%, respectively. HFD feeding reduced the activity of malic enzyme by 62%. Whereas feeding of CB and CAP along with HFD showed a tendency to counter this decrease in the activity of malic enzyme (not statistically significant), the combination of CB and CAP increased the activity of malic enzyme by 29% compared to HFD control ($p < 0.5$). In the basal group, all three dietary interventions, namely, CB, CAP, and their combination, brought about 26.6, 63.8, and 61.2% decreases in its activity, respectively.

HFD feeding resulted in a reduction in the activity of hepatic glucose-6-phosphate dehydrogenase (62% decrease) (Table 4). Inclusion of CB, CAP, and their combination along with HFD countered this decrease by 17, 23, and 49%, respectively. Thus, the effect of the combination of CB and CAP on the activity of this NADPH-producing lipogenic enzyme was additive. In the basal diet groups, CB and CAP independently brought about around a 51% reduction, whereas the combination brought a 53.6% reduction. Ingestion of HFD decreased the activity of hepatic 6-phosphogluconate dehydrogenase by 70%. Dietary CB and CAP enabled recoveries of the activity of this enzyme to extents of 19 and 23%, whereas the combination of the two recovered it by 40%, thus amounting to an additive effect. In rats fed basal diet, the activity of hepatic 6-phosphogluconate dehydrogenase was reduced by 36.5, 67.2, and 66% as a result of feeding CB, CAP, and their combination, respectively.

Influence of Dietary Cluster Beans and Capsaicin on Dietary Fat Absorption. Fecal excretion of triglycerides increased significantly by CB feeding both in high-fat-fed and in normal situations (Table 5). HFD feeding brought about a 6-fold increase in fecal triglyceride excretion, this higher excretion being necessitated by higher intake (30% fat in the diet). Dietary CB enhanced the excretion of triglycerides in the feces by 44%, whereas capsaicin brought about 56% increased excretion of the same. Inclusion of the combination of CB and capsaicin yielded a 90% increase in fecal triglycerides, amounting to an additive effect. In basal diet groups, CB-fed animals showed a 134% increase in fecal triglyceride excretion, whereas capsaicin produced a 191% increase. Combination of the two enhanced fecal triglycerides by 288%. There was an observable decrease in fat absorption by dietary CB and capsaicin. Rats fed HFD enriched with CB had a marginal decrease in fat absorption (7.5% decrease), whereas capsaicin feeding alone decreased the absorption of dietary fat by 13.4%, and the combination of the two produced a 14.6% decrease in the same ($p < 0.05$). In the rats fed basal diet, a significant 18.7% lesser absorption of triglycerides was observed in the case of combination of CB and capsaicin.

■ DISCUSSION

Obesity is known to result from an imbalance between energy intake and energy expenditure; consequently, prevention of excess fat deposition is a strategy for the prevention of obesity and metabolic disorders associated with it. Over the past few decades, significant progress has been made in understanding the role of dietary fiber in the promotion of health and reduction of obesity-related disease risk. Studies have indicated that consumption of chilli-containing meals increases energy expenditure and fat oxidation due to its active principle, capsaicin. Capsaicin exerts a lipotrophic effect similar to choline in rats and decreases serum, myocardial, and aortic cholesterol level in rats.[20] The present investigation explored the potential health benefits of consuming tender cluster bean as a source of soluble dietary fiber and also its antiobesity effect, both alone and in combination with capsaicin. Similar to the lowered adipose triglycerides as seen in the present investigation by feeding cluster bean and capsaicin, guar gum hydrolysate has been reported to reduce the epididymal adipose tissue weight.[21] Perirenal adipose tissue weight was decreased in rats by inclusion of capsaicin in the diet.[22] The antilipogenic effects and hence antiobesity potential of capsaicin have recently been well demonstrated by several proteomic studies in experimental rats.[23,24]

A high-fat diet did not affect the feed intake in animals in the present study. A similar observation has been reported, where HFD did not alter the feed intake in rats.[25] The weight gain was reduced by CB and capsaicin independently and in combination in both basal and HFD groups. CB and capsaicin, individually and in combination, suppressed body weight but not feed intake. In experimental studies, where oral capsaicin was provided to rodents as 0.014% of the diet, a dose equivalent to that ingested by rural Thai people, there were significant 24 and 29% reductions in the weight of perirenal fat, without an effect on total caloric intake.[26]

This study established that consumption of fiber-rich vegetables and the pungent spice compound together has a better antihypercholesterolemic effect. The present investigation showed that CB has a tendency to correct the altered serum and liver lipid profile, especially to lower total and LDL-cholesterol in high-fat-fed condition. We have recently reported that dietary tender cluster beans produce a significant hypolipidemic effect in hypercholesterolemic rats, and dietary garlic potentiated this hypocholesterolemic effect of cluster beans.[27] Our study also found improvement in health indices such as atherogenicity index and cholesterol/phospholipid ratio in serum and liver. Besides, it was inferred that CB as a source of dietary fiber has the potential of improving cardiovascular health. An improved atherogenicity index has been reported by feeding 10% guar gum in the atherogenic diet of rats.[28]

Feeding HFD increased the liver lipids significantly. CB and capsaicin, individually or in combination, significantly countered it. This action of CB to suppress hepatic cholesterol in high-fat-fed as well as normal conditions establishes the utility of fiber in diverting cholesterol into bile acids and facilitating their excretion, which results in reduction of cholesterol in body tissues. We have recently observed that the 6-fold elevation in hepatic cholesterol in hypercholesterolemic animals was also countered by dietary CB and garlic, their combination producing a synergistic reduction.[27] Countering of the increase in hepatic triglycerides was also additive by the combination of dietary CB and garlic. Rideout et al. have reported that the increased hepatic demand for cholesterol from the guar gum induced reduction in hepatic free cholesterol pool can be met by an increased uptake of lipoprotein cholesterol from plasma, the release of free cholesterol from intracellular cholesteryl ester and membrane cholesterol, or increasing hepatic cholesterol synthesis.[28] Inclusion of 0.014% capsaicin in the diet reduced serum triglycerides in high-fat-fed rats as reported.[22] Similarly, administration of capsaicin to hypertriglyceridemic rats resulted in lowered serum and liver lipids.[29] Capsaicin administered to hypertriglyceridemic rats lowered hepatic triglyceride significantly.[30]

There appears to be a tremendous potential in CB and capsaicin to lower and diverge body stores of triglycerides through feces and bring about beneficial a decrease in its content in body tissues. Our study revealed that consumption of CB enhanced fecal triglyceride excretion and decreased absorption of dietary fat; both of these work toward one common end, that is, lowering triglycerides in body tissues. One of the primary actions of soluble fiber in the intestine is to reduce dietary fat and cholesterol uptake.[31] The interference mechanisms include direct binding of cholesterol to the soluble fiber within the intestinal lumen and interference with the diffusion of luminal cholesterol toward the epithelial cell surface.[31] The cholesterol-lowering effect of guar gum is mediated by an accelerated fecal excretion of steroids and biliary secretion of bile acids;[32] the increase in fecal loss of bile acids resulting from a reduction in the enterohepatic recirculation of bile acids stimulates the liver to produce more bile acids from cholesterol, thus reducing the hepatic free cholesterol concentration. Fat absorption was significantly reduced by CB independently or along with capsaicin. The extent of dietary triglyceride absorption in rats fed test diets was markedly decreased, which is consistent with the decrease in triglycerides in tissues and fecal excretion of unabsorbed triglycerides. Reducing fat absorption is an effective way to suppress body weight gain. In the present study, we report this observation on a fiber-rich vegetable that interrupts fat absorption and diverts it out of the body.

Capsaicin, the pungent principle of red pepper, has been reported to reduce adiposity in rats, which can be partly explained by the enhancing effects on energy and lipid metabolism via catecholamine secretion from the adrenal medulla through sympathetic activation of the central nervous system. An increase in diet-induced thermogenesis and a decrease in respiratory quotient immediately after a meal containing red pepper/capsaicin have been reported.[33] Decreased appetite, decreased food intake, and increased energy expenditure have been shown after consumption of red pepper.[34]

Adipose tissue and liver are the two main sites of de novo lipogenesis, the synthesis of fatty acid molecules from nonlipid substrates.[35] ATP-citrate lyase, which provides the substrate acetyl-CoA for fatty acid synthesis in the cytoplasm, and the key enzymes that provide the supply of NADPH in cytoplasm required for fatty acid synthesis (G6PDH, 6PGDH, and malic enzyme), represent the lipogenic enzymes. The activity of lipogenic enzymes was decreased in HFD-fed rats, which may be an indication of the body's homeostasis, wherein lipogenesis in the body is diminished due to availability of excess dietary fat. CB and capsaicin by virtue of their independent mechanisms cause decreased fat absorption and a higher excretion of fat in the feces and, hence, reduced fat accumulation in body. This in turn revived the activity of lipogenic enzymes in the body. HFD reduced the activity of lipogenic enzymes (glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, and malic enzyme) which produce NADPH required for lipogenesis. The activities of these dehydrogenases increase during elevated lipid synthesis, whereas inclusion of fat in the diet leads to a marked reduction in the rate of fatty acid synthesis and the level of lipogenic enzymes.[35] ATP-citrate lyase is the primary enzyme responsible for the synthesis of cytosolic acetyl-CoA in many tissues; acetyl-CoA serves several important biosynthetic pathways including lipogenesis and cholesterogenesis.[35]

The activity of hormone-sensitive lipase (HSL) that causes lipolysis of adipose triglycerides is associated with the release of adipose fatty acids into circulation and was found to be enhanced by diets enriched with CB, capsaicin, or their combination. This mobilizes more and more accumulated triglycerides, favoring a more rapid turnover of tissue lipids. Inhibition of HSL activity by extract from dried roots of *Salacia reticulate* and inhibition of fat accumulation in adipose tissue in a high-fat-fed situation have been reported.[36]

Thus, it may be inferred that dietary CB is a potential antiobesity agent that could check the gain in body weight through impeding fat absorption and its accumulation in adipose depot and enhancing fat excretion to an apreciable extent. CB and capsaicin in combination acted almost additively in decreasing the body weight gain in high-fat-fed rats and brought a significant decrease in weight gain in the animals fed basal diet. Adipose tissue weight was decreased by inclusion of CB or capsaicin in the diet. The decreasing effect of the combination on adipose triglyceride was greater than their individual effects. THe combination of CB and capsaicin along with HFD produced an additive effect in increasing the activity of HSL in the adipose tissue, which facilitates mobilization of adipose fat by these dietary interventions in high-fat-fed animals. Increased fecal triglycerides by the combination of CB and capsaicin was additive. We observed an impressive capability of fiber-rich CB and capsaicin to lower triglycerides in serum, liver, and adipose with a concomitant higher fecal excretion. Consistent lowering of triglycerides in various tissues of the body ensures the antiobesity role of CB, which is further enhanced by capsaicin. Dietary CB and capsaicin brought about a significant hypolipidemic effect both independently as well as in combination in hypertriglyceridemic rats; the effect, although not additive, was more than the individual effects. These results suggest the potential of fiber-rich CB in improving cardiovascular health. The combination of CB and capsaicin lowered hepatic cholesterol and triglycerides by an extent that amounted to an additive effect of the two ingredients.

The effects of cluster beans and capsaicin seemed quite similar on most parameters examined in this investigation in connection with weight control. These two exert their influences potentially by very different mechanisms of action, that is, CB by altering the gut environment and capsaicin by thermogenesis. The effect of these two interventions appears to be complementary in terms of the extent of the desired action.

The freeze-dried tender cluster bean powder used in our study contained total polyphenols to an extent of 398 mg/100 g (data not shown). When included in the diet at 15 g/100 g, the total polyphenol concentration in the diet would be 60 mg/100 g, compared to 1.83 g soluble fiber and 3.87 g insoluble fiber/100 g diet. Hence, it is less likely that components other than dietary fiber such as polyphenols may have contributed to the observed hypolipidemic and weight-reducing action of tender cluster beans.

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Funding

S.P. is thankful to the University Grants Commission, New Delhi, for the award of a Senior Research Fellowship.

Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Pittler, M. H.; Ernst, E. Dietary supplements for body-weight reduction: a systematic. *Am. J. Clin. Nutr.* **2004**, *79*, 529−536.

(2) Li, Z.; Bowerman, S.; Heber, D. Health ramifications of the obesity epidemic. *Surg. Clin. North Am.* **2005**, *85*, 681−701.

(3) Schulz, M.; Nöthlings, U.; Hoffmann, K.; Bergmann, M. M.; Boeing, H. Identification of a food pattern characterized by high-fiber and low-fat food choices associated with low prospective weight change in the EPIC-potsdam cohort. *J. Nutr.* **2005**, *135*, 1183−1189.

(4) Newby, P. K.; Tucker, K. L.; Wolk, A. Risk of overweight and obesity among semivegeterian, lactoovo-vegetarian and vegan women. *Am. J. Clin. Nutr.* **2005**, *81*, 1267−1274.

(5) Delzenne, N. M.; Cani, P. D. A place for dietary fibre in the management of the metabolic syndrome. *Curr. Opin. Clin. Nutr. Metab. Care* **2005**, *8*, 636−640.

(6) Butt, M. S.; Shahzadi, N.; Sharif, M. K.; Nasir, M. Guar gum: A miracle therapy for hypercholesterolemia, hyperglycemia and obesity. *Crit. Rev. Food Sci. Nutr.* **2007**, *47*, 389−396.

(7) Srinivasan, K.; Sambaiah, K.; Chandrasekhara, N. Spices as beneficial hypolipidemic food adjuncts: a review. *Food Rev. Int.* **2004**, *20*, 187−220.

(8) Asp, N.; Johanson, G.; Hallmer, H.; Siljestron, M. Rapid enzymatic assay of insoluble and soluble dietary fiber. *J. Agric. Food Chem.* **1983**, *31*, 476−482.

(9) Fletcher, M. J. A colorimetric method for estimating serum triglycerides. *Clin. Chim. Acta* **1968**, *22*, 393−397.

(10) Folch, J.; Lees, M.; Sloane-Stanley, G. H. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **1957**, *226*, 497−509.

(11) Searcy, R. L.; Bergquist, L. M. A new colour reaction for quantification of serum cholesterol. *Clin. Chim. Acta* **1960**, *8*, 192−197.

(12) Warnick, G. R.; Albers, J. J. A comprehensive evaluation of the heparin-manganese precipitation procedure for estimating high density lipoprotein cholesterol. *J. Lipid Res.* **1978**, *19*, 65−76.

(13) Stewart, J. C. M. Colorimetric determination of phospholipids with ammonium ferrothio cyanate. *Anal. Biochem.* **1980**, *104*, 10−14.

(14) Takeda, Y.; Suzuki, F.; Inoue, H. ATP-citrate lyase (citrate cleavage enzyme). *Methods Enzymol.* **1969**, *13*, 641−644.

(15) Deutsch, J. Glucose-6-phosphate dehydrogenase. In *Methods of Enzymatic Analysis*; Bergmeyer, H. U., Ed.; Academic Press: New York, 1983; Vol. 3, pp 176−183.

(16) King, J. 6-Phosphogluconate dehydrogenase. In *Methods of Enzymatic Analysis*; Bergmeyer, H. U., Ed.; Academic Press: New York, 1974; Vol. 2, pp 632−635.

(17) Ochoa, S. Malic enzyme. *Methods Enzymol.* **1955**, *1*, 739−753.

(18) Khoo, J. C.; Steinberg, D. Hormone-sensitive triglyceride lipase from rat adipose tissue. *Methods Enzymol.* **1975**, *35*, 181−183.

(19) Belfrage, P.; Vaughan, M. Simple liquid-liquid-partition system for isolation of labeled oleic acid from mixtures with glycerides. *J. Lipid Res.* **1969**, *10*, 341−344.

(20) Sambaiah, K.; Satyanarayana, M. N. Lipotrope-like activity of red pepper. *J. Food Sci. Technol.* **1982**, *19*, 30−31.

(21) Suzuki, T.; Hara, H. Ingestion of guar gum hydrolysate, a soluble and fermentable nondigestible saccharide, improves glucose intolerance and prevents hypertriglyceridemia in rats fed fructose. *J. Nutr.* **2004**, *134*, 1942−1947.

(22) Kawada, T.; Hagihara, K.; Iwai, K. Effects of capsaicin on lipid metabolism in rats fed high fat diet. *J. Nutr.* **1986**, *116*, 1272−1278.

(23) Joo, J. I.; Kim, D. H.; Choi, J. W.; Yun, J. W. Proteomic analysis for antiobesity potential of capsaicin on white adipose tissue in rats fed with a high-fat diet. *J. Proteome Res.* **2010**, *9*, 2977−2987.

(24) Choi, J. W.; Hwang, H. S.; Kim, D. H.; Joo, J. I.; Yun, J. W. Proteomic analysis of liver proteins in rats fed with a high-fat diet in response to capsaicin treatments. *Biotechnol. Bioprocess Eng.* **2010**, *15*, 534−544.

(25) Yoshikawa, M.; Ninomiya, K.; Shimoda, H.; Nishida, N.; Matsuda., H. Hepatoprotective and antioxidative properties of *Salacia reticulata*: preventive effects of phenolic constituents on CCl_4-induced liver injury in mice. *Biol. Pharm. Bull.* **2002**, *25*, 72−76.

(26) Donnerer, J.; Amann, R.; Schuligoi, R.; Lembeck, F. Absorption and metabolism of capsaicinoids following intragastric administration in rats. *Naunyn−Schmiedeberg's Arch. Pharmacol.* **1990**, *342*, 357−361.

(27) Pande, S.; Srinivasan, K. Potentiation of the hypolipidemic influence of dietary tender cluster bean (*Cyamopsis tetragonoloba*) by garlic in cholesterol fed rats. *Food Chem.* **2012**, *133*, 798−805.

(28) Rideout, T. C.; Yuan, Z.; Bakovic, M.; Liu, Q.; Li, R. K.; Mine, Y.; Fan, M. Z. Guar gum consumption increases hepatic nuclear SREBP2 and LDL receptor expression in pigs fed an atherogenic diet. *J. Nutr.* **2007**, *137*, 568−572.

(29) Tani, Y.; Fujioka, T.; Sumioka, M.; Furuichi, Y.; Hamada, H.; Watanabe, T. Effects of capsinoid on serum and liver lipids in hyperlipidemic rats. *J. Nutr. Sci. Vitaminol.* **2004**, *50*, 351−355.

(30) Kempaiah, R. K.; Srinivasan, K. Beneficial influence of dietary curcumin, capsaicin and garlic on erythrocyte integrity in high-fat fed rats. *J. Nutr. Biochem.* **2006**, *17*, 471−478.

(31) Schneeman, B. O. Fiber, inulin and oligofructose: similarities and differences. *J. Nutr.* **1999**, *129*, 1424−1427.

(32) Moundras, C.; Behr, S. R.; Remesy, C.; Demigne, C. Fecal losses of sterol and bile acid induced by feeding rats guar gum are due to greater pool size and liver bile acid secretion. *J. Nutr.* **1997**, *12*, 1068−1076.

(33) Yoshioka, M.; St-Pierre, S.; Suzuki, M.; Tremblay, A. Effects of red pepper added to high-fat and high-carbohydrate meals on energy metabolism and substrate utilization in Japanese women. *Br. J. Nutr.* **1998**, *80*, 503−510.

(34) Yoshioka, M.; St-Pierre, S.; Drapeau, V.; Dionne, I.; Doucet, E.; Suzuki, M.; Tremblay, A. Effects of red pepper on appetite and energy intake. *Br. J. Nutr.* **1999**, *82*, 115−123.

(35) Nelson, D. L.; Cox, M. M. In *Lehninger − Principles of Biochemistry*, 5th ed.; W. H. Freeman: New York, 2008.

(36) Yoshikawa, M.; Shimoda, H.; Nishida, N.; Takada, M.; Matsuda, H. *Salacia reticulata* and its polyphenolic constituents with lipase inhibitory and lipolytic activities have mild antiobesity effects in rats. *J. Nutr.* **2002**, *132*, 1819−1824.