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A Freshwater Clam (*Corbicula fluminea*) Extract Reduces Cholesterol Level and Hepatic Lipids in Normal Rats and Xenobiotics-Induced Hypercholesterolemic Rats

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We investigated whether a freshwater clam (*Corbicula fluminea*) extract (FCE) could improve cholesterol metabolism and hepatic lipids accumulation in rats fed xenobiotics such as chloretone. Feeding chloretone resulted in hypercholesterolemia and fatty liver. An increase in serum cholesterol, high density lipoproteins (HDL) in particular, after intake of chloretone was observed. Serum cholesterol was decreased by supplementation with FCE. Accumulation of the hepatic lipids including triacylglycerol, cholesterol, and phospholipid was significantly suppressed by supplementation with FCE. The excretion of neutral and acidic sterols into the feces was enhanced by FCE. The hepatic gene expression of cholesterol 7α-hydroxylase was enhanced in rats fed a FCE-containing diet. Apolipoprotein A-I gene expression in the liver, which is a major apolipoprotein of HDL, was suppressed by FCE. These results demonstrated that FCE reduced cholesterol level and hepatic lipids in normal rats and hypercholesterolemic rats fed chloretone.

KEYWORDS: Hypercholesterolemia; fatty liver; freshwater clam extract; xenobiotics; chloretone

INTRODUCTION

The freshwater clam is a popular edible bivalve in Asia. It has been reported that freshwater clams are effective against hypercholesterolemia induced by feeding a high-cholesterol diet (*1*−*3*). Shellfish contains several lipophilic plant sterols (*1*−*5*). These plant sterols have been reported to effectively reduce cholesterol absorption from the intestine (*2, 3, 5*). Iritani et al. (*2, 3*) suggested that minced freshwater clam had a hypocholesterolemic effect by inhibiting cholesterol absorption by plant sterols in rats fed a high cholesterol diet. However, the amount of plant sterols in freshwater clam extract (FCE) is not enough to exert a hypercholesterolemic action (*1*). Recently we found that FCE showed hypocholesterolemic effects by increasing the fecal excretion of bile acids and induction of cholesterol 7α-hydroxylase (CYP7A1; EC1.14.13.7) gene expression in rats fed a high-cholesterol diet (*1*).

The rat model for hypercholesterolemia induced by feeding a high-cholesterol diet has been extensively studied (*1*−*3, 6, 7*). We have also used the exogenous hypercholesterolemic model in order to observe an ameliorative effect of FCE on the hypercholesterolemia (*1*). However, this model is not always appropriate to investigate cholesterol metabolism because hepatic cholesterogenesis is inhibited by excess exogeneous cholesterol (*8*). In some cases of hypercholesterolemic humans, cholesterogenesis seems to play an important role in the development of hypercholesterolemia.

It is known that administration of xenobiotics, such as chloretone, pentobarbital, polychlorinated biphenyls (PCB), 2,6-di-*tert*-butyl-*p*-cresol (BHT), and 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT), to animals causes hypercholesterolemia with an elevation of HDL-cholesterol and apolipoprotein (apo) A-I (*9, 10*), accumulation of hepatic lipids (*11, 12*), an activation of hepatic drug metabolizing enzymes (*13*), and an increase in ascorbic acid levels in urine and tissues (*11, 14*). It is thought that hypercholesterolemia induced by xenobiotics is mainly due to the stimulation of cholesterogenesis with both the activation of hepatic 3-hydroxy-3-methyl coenzyme A (HMG-CoA) reductase (EC 1.1.1.34) (*15, 16*) and the induction of its gene expression (*17*). Additionally, the development of a fatty liver by exposure to xenobiotics was caused by the induction of the gene expression for lipogenic enzymes (*18*). Therefore, the xenobiotics-induced hypercholesterolemia should be a good model for humans with endogenous hypercholesterolemia. In

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Table 1. Amino Acid Profiles (mg/g of nitrogen) of Freshwater Clam Extract (FCE) and Casein

	FCE[a]	casein[b]
aspartic acid	696	460
threonine	312	270
serine	295	340
glutamic acid	951	1400
glycine	360	120
alanine	349	200
valine	330	440
cysteine	49	32
methionine	152	200
isoleucine	302	360
leucine	486	620
tyrosine	257	370
phenylalanine	266	340
histidine	180	200
lysine	468	530
tryptophan	84	84
arginine	440	240
proline	275	750

[a] FCE is freshwater clam extract. [b] The data of casein was quoted from *Standard of Food Composition in Japan*, 4th ed (*17*).

the present study, we used chloretone as the xenobiotic, which is widely employed as an antiseptic agent. Thus, we investigated whether FCE affected hypercholesterolemia induced by xenobiotics in rats.

MATERIALS AND METHODS

Preparation of the Freshwater Clam Extract. FCE was prepared with the procedure described in a previous paper (*1*). Freshwater clams (*Corbicula fluminea*) were steamed until the shells opened, and the edible portions were removed. The edible portion was minced, extracted with boiling water, and then filtered through the 80 mesh filter. The filtrate was spray-dried for use as the FCE. The yield of FCE from raw material was approximately 1.5% (w/w). FCE contains not only soluble materials but also approximately 35% insoluble materials. The proximate compositions of FCE (59.8 g of protein, 19.2 g of carbohydrate, 4.8 g of moisture, 10.6 g of crude fat and 5.6 g of ash) were contained in 100 g of the powder. Amino acid profiles of FCE and casein (*19*) are shown in **Table 1**. It was notable that glycine in FCE was three times higher than that in the casein. The amount of phytosterols in FCE was described in our previous paper (*1*). Briefly, the amounts of campesterol, brassicasterol, stigmasterol, and β-sitosterol in 1 g of FCE were 3.31, 1.80, 1.42, and 0.33 μmol, respectively.

Animals and Diets. Male Wistar rats, aged 4 weeks, with a body weight of about 100 g were obtained from Japan SLC (Hamamatsu, Japan). The animals were maintained at 23 °C with a 12 h light (8:00−20:00) and dark (20:00−8:00) cycle. To accustom the rats to the experimental conditions, they were initially fed a commercial stock diet (5L37; Japan SLC, Inc.) for 5 days and then fed a 20% casein diet for 4 days before being divided into four groups of six animals each. The compositions of the experimental diets are shown in **Table 2**. The animals were fed a basal diet (control group), a basal diet supplemented with 30 g of FCE per 100 g of the diet (FCE group), a basal diet supplemented with 0.3 g of chloretone per 100 g of diet (Chloretone group), and a basal diet supplemented with 0.3 g of chloretone and 30 g of FCE per 100 g of diet (Chloretone + FCE group) for 2 weeks. FCE was added to the basal diet at the expense of casein, sucrose, and α-cornstarch. The dietary protein levels of all experimental diets were the same. During the study, rats were maintained in individual stainless steel cages and had free access to the experimental diets and water. Feces were collected over the final 3 days of the experimental period and used for determining fecal neutral sterols and bile acids.

The rats in all groups were anesthetized with diethyl ether and killed at 22:00, after 4 h of fasting, on the last day of the experimental period. Blood was collected by cardiac puncture for analysis of the serum lipids. A liver sample from each rat was removed rapidly and immediately frozen and stored for analysis of the hepatic lipids and the gene expression linked to cholesterol synthesis and metabolism. The experimental procedures used in this study met the guidelines of the Animal Care and Use Committee of Oita University.

Table 2. Compositions (g/kg) of the Experimental Diets

	control group[a]	FCE group[a]	chloretone group[a]	chloretone + FCE group[a]
casein	200.0		200.0	
FCE[b]		300.0		300.0
sucrose	235.0	202.0	234.0	201.0
corn oil	50.0	50.0	50.0	50.0
α-corn starch	470.0	403.0	468.0	401.0
AIN-76 mineral mixture[c]	35.0	35.0	35.0	35.0
AIN-76 vitamin mixture[c]	10.0	10.0	10.0	10.0
chloretone			3.0	3.0

[a] Control group, basal diet; FCE group, FCE-supplemented basal diet; chloretone group, chloretone-supplemented basal diet; chloretone + FCE group, chloretone and FCE-supplemented basal diet. [b] FCE means freshwater clam extract. [c] Supplied by Oriental Yeast, Tokyo, Japan.

Biochemical Analyses. Serum total cholesterol and HDL-cholesterol were determined using commercial kits (T-CHO: Kainos Laboratories, Tokyo, HDL-cholesterol-test: Wako Pure Chemical Industries, Osaka, Japan). About 2.5 g of liver was homogenized, and the lipids were extracted with a chloroform/methanol mixture (2:1, v/v) as described by Folch et al. (*20*). Total lipids in the liver were determined gravimetrically. The concentrations of hepatic cholesterol, triglyceride, and phospholipid in the lipids extracts were determined using a commercial kit (T-CHO, TG-EN: Kainos Laboratories, Tokyo, phospholipids C-Test: Wako Pure Chemical Industries, Osaka, Japan). Fecal sterols were extracted by the method of Delaney et al. (*21*). Fecal neutral sterols were analyzed as the trimethylsilyl ester using gas chromatography/mass spectrometry (GC 6890 equipped with 5973MSD and a 30 m × 0.25 mm HP-5 ms capillary column; Agilent) with 5α-cholestane as the internal standard. The injector and detector temperatures were set at 300 and 230 °C, respectively. The initial column temperature was 245 °C; it was held for 2 min and then increased to 300 °C at a rate of 2 °C/min. Fecal bile acids were enzymatically determined by the method of Sheltawy and Losowsky (*22*), with lithocholic acid used as the standard.

Total RNA was isolated according to the method described by Chomczynski and Sacchi (*23*), and 20 μg of total RNA was subjected to Northern blot hybridization. The cDNA clones of hamster HMG-CoA reductase, rat CYP7A1, rat liver X receptor (LXR), rat small heterodimer partner (SHP), rat hepatocyte nuclear factor 4 (HNF-4), rat apo A-I, and mouse apo E were labeled with the Megaprine DNA labeling system (Amersham, Tokyo, Japan) and used for hybridization. Specific hybridization was quantified with an image analyzer (BAS 2000, Fuji Film, Tokyo, Japan). The apo E mRNA level was not affected by any treatment employed in this study (data not shown), so we used it as a normalization standard (*24*, *25*).

Statistical Analysis. The significance of differences among values was analyzed by a two-way analysis of variance (ANOVA). When the interaction (chloretone × FCE) was significant ($p < 0.05$), a Student's t test was performed. The criterion for significance was $p < 0.05$ or $p < 0.01$, as specified. Values in the text are reported as means ± SEM.

RESULTS

The supplementation of FCE significantly increased the body weight gain of the rats untreated with chloretone (Student's t test; control group vs FCE group, $p < 0.05$) (**Table 3**). Treatment with chloretone significantly increased body weight gain (Student's t test; control group vs chloretone group, $p < 0.01$), and supplementation with FCE significantly suppressed that increase (Student's t test; chloretone group vs chloretone + FCE group, $p < 0.05$). Intake of FCE decreased the relative liver weight ($p < 0.01$), whereas chloretone increased it ($p < 0.01$).

The serum cholesterol levels were increased by supplementation with chloretone ($p < 0.01$) (**Table 4**). FCE induced the

Table 3. Effects of Freshwater Clam Extract (FCE) on Body and Relative Liver Weights in Rats Fed a Control or Chloretone-Containing Diet for 2 Weeks[a]

					ANOVA		
	control group[b]	FCE group[b]	chloretone group[b]	chloretone + FCE group[b]	chloretone	FCE[c]	interaction
initial body weight (g)	105 ± 1	105 ± 1	105 ± 1	105 ± 1	NS[d]	NS	NS
final body weight (g)	182 ± 2	187 ± 2	191 ± 3**	185 ± 2	NS	NS	0.05
body weight gain (g for 2 weeks)	76 ± 2	82 ± 2*	86 ± 2**	80 ± 2[#]	0.05	NS	0.01
relative liver weight (g/100 g of body weight)	4.37 ± 0.16	4.11 ± 0.09	6.26 ± 0.12	5.61 ± 0.14	0.01	0.01	NS

[a] Each value is the mean ± SEM for six rats in each dietary group. [b] Control group, basal diet; FCE group, FCE-supplemented basal diet; chloretone group, chloretone-supplemented basal diet; chloretone + FCE group, chloretone and FCE-supplemented basal diet. [c] FCE means freshwater clam extract. [d] Statistical significance of differences among values was analyzed by two-way ANOVA. When the interaction (chloretone × FCE) was significant, Student's *t* test was performed. NS, not significant ($p > 0.05$). * and ** indicate that these values differed significantly at $p < 0.05$ or <0.01 from the values of the control group. [#] and [##] indicate that these values differed significantly at $p < 0.05$ or <0.01 from the values of the chloretone group.

Table 4. Effects of Freshwater Clam Extract (FCE) on Serum Cholesterol and HDL-cholesterol (mg/dL) in Rats Fed a Control or Chloretone-Containing Diet for 2 Weeks[a]

					ANOVA		
	control group[b]	FCE group[b]	chloretone group[b]	chloretone + FCE group[b]	chloretone	FCE[c]	interaction
total cholesterol	126 ± 6	60.7 ± 2.2	185 ± 8	107 ± 7	0.01[d]	0.01	NS
HDL-cholesterol	91.8 ± 3.0	50.8 ± 1.7	128 ± 4	83.0 ± 4.8	0.01	0.01	NS

[a] Each value is the mean ± SEM for six rats in each dietary group. [b] Control group, basal diet; FCE group, FCE-supplemented basal diet; chloretone group, chloretone-supplemented basal diet; chloretone + FCE group, chloretone and FCE-supplemented basal diet. [c] FCE means freshwater clam extract. [d] Statistical significance of differences among values was analyzed by two-way ANOVA. When the interaction (chloretone × FCE) was significant, Student's *t* test was performed. NS, not significant ($p > 0.05$).

Table 5. Effects of Freshwater Clam Extract (FCE) on Hepatic Lipids (mg/g liver) of Rats Fed a Control or Chloretone-Containing Diet for 2 Weeks[a]

					ANOVA		
	control group[b]	FCE group[b]	chloretone group[b]	chloretone + FCE group[b]	chloretone	FCE[c]	interaction
total lipids	74.8 ± 10.2	56.3 ± 1.1	206 ± 12**	64.0 ± 0.9[##]	0.01[d]	0.01	0.01
triacylglycerol	43.8 ± 14.5	15.7 ± 1.2*	170 ± 10**	14.0 ± 0.8[##]	0.01	0.01	0.01
cholesterol	3.62 ± 0.26	3.05 ± 0.07*	6.01 ± 0.23**	3.22 ± 0.10[##]	0.01	0.01	0.01
phospholipid	23.7 ± 0.7	24.1 ± 0.2	27.1 ± 0.6**	29.9 ± 0.3[##]	0.01	0.01	0.05

[a] Each value is the mean ± SEM for six rats in each dietary group. [b] Control group, basal diet; FCE group, FCE-supplemented basal diet; chloretone group, chloretone-supplemented basal diet; chloretone + FCE group, chloretone and FCE-supplemented basal diet. [c] FCE means freshwater clam extract. [d] Statistical significance of differences among values was analyzed by two-way ANOVA. When the interaction (chloretone × FCE) was significant, Student's *t* test was performed. NS, not significant ($p > 0.05$). * and ** indicate that these values differed significantly at $p < 0.05$ or <0.01 from the values of the control group. [##] indicates that these values differed significantly at $p < 0.01$ from the values of the chloretone group.

reduction of the serum cholesterol level ($p < 0.01$). HDL-cholesterol in the serum was also increased by chloretone intake ($p < 0.01$) and decreased by FCE ($p < 0.01$) (**Table 4**).

The hepatic lipids, in particular the total lipids and triglyceride, were significantly increased by supplementation with chloretone (Student's *t* test; control group vs chloretone group, $p < 0.01$) (**Table 5**). However, FCE completely inhibited the increment of hepatic lipids by chloretone, in spite of the fact that phospholipid was unaffected (Student's *t* test; chloretone group vs chloretone + FCE group, $p < 0.01$). Also, the hepatic concentration of cholesterol was significantly elevated by chloretone (Student's *t* test; control group vs cholretone group, $p < 0.01$) (**Table 5**). However, the intake of FCE significantly restrained the elevation of hepatic cholesterol by chloretone (Student's *t* test; chloretone group vs chloretone + FCE group, $p < 0.01$).

The fecal excretions of neutral sterols (cholesterol, coprostanol, and coprostanone) were not altered by supplementation with chloretone (Student's *t* test; control group vs chloretone group, $p > 0.05$) (**Table 6**). On the other hand, the supplementation of FCE to the control diet significantly increased neutral sterols in the feces (Student's *t* test; control group vs FCE group, $p < 0.01$). Furthermore, in the chloretone-treated groups, supplementation of FCE significantly increased fecal neutral sterols (Student's *t* test; chloretone group vs chloretone + FCE group, $p < 0.01$). The bile acids excretion into the feces was elevated by FCE intake ($p < 0.01$). However, there were no effects of chloretone on the excretion of bile acids into the feces ($p > 0.05$).

The hepatic mRNA levels of HMG-CoA reductase, CYP7A1, LXR, HNF-4, SHP, and apo A-I were also measured. It was reported that dietary PCB induced hypercholesterolemia in rats due to the induction of the hepatic HMG-CoA reductase gene (*17*). Although dietary chloretone tended to increase the hepatic mRNA level of HMG-CoA reductase, treatment with FCE did not change that (**Table 7**). On the other hand, the hepatic level of CYP7A1 mRNA was increased by chloretone ($p < 0.05$), and FCE caused a larger increment in CYP7A1 mRNA levels ($p < 0.01$). Although an increase in the HNF-4 mRNA level by treatment with FCE was observed in rats fed a high-cholesterol diet (*1*), the mRNA level was not altered by FCE treatment to rats fed a chloretone-containing diet. The mRNA levels of LXR were not altered by FCE and chloretone. The level of SHP mRNA was increased by dietary chloretone ($p < 0.05$). On the other hand, FCE did not change the SHP mRNA level. It has been reported that dietary xenobiotics such as PCB can induce apo A-I gene expression (*26*). However, chloretone treatment did not change apo A-I gene expression. Interestingly, FCE addition to the food strongly suppressed the hepatic apo A-I gene expression ($p < 0.01$).

Table 6. Effects of Freshwater Clam Extract (FCE) on Excretion of Sterols (μmol/3 days) into the Feces in Rats Fed a Control or Chloretone-Containing Diet for 2 Weeks[a]

					ANOVA		
	control group[b]	FCE group[b]	chloretone group[b]	chloretone + FCE group[b]	chroletone	FCE[c]	interaction
cholesterol	0.81 ± 0.15	25.0 ± 5.7	1.31 ± 0.17	23.1 ± 4.5	NS[d]	0.01	NS
coprostanol	4.49 ± 0.38	13.8 ± 3.5	7.00 ± 1.13	42.6 ± 10.5[##]	0.05	0.01	0.05
coprostanone	0	7.45 ± 1.73	0	9.42 ± 2.10	NS	0.01	NS
neutral sterols[e]	5.30 ± 0.51	46.2 ± 3.8**	8.31 ± 1.27	75.2 ± 10.4[##]	0.01	0.01	0.05
bile acids	37.5 ± 2.6	303 ± 36	42.9 ± 3.7	275 ± 32	NS	0.01	NS

[a] Each value is the mean ± SEM for six rats in each dietary group. [b] Control group, basal diet; FCE group, FCE-supplemented basal diet; chloretone group, chloretone-supplemented basal diet; chloretone + FCE group, chloretone and FCE-supplemented basal diet. [c] FCE means freshwater clam extract. [d] Statistical significance of differences among values was analyzed by two-way ANOVA. When the interaction (chloretone × FCE) was significant, Student's *t* test was performed. NS, not significant ($p > 0.05$). ** indicates that these values differed significantly at $p < 0.01$ from the values of the control group. ## indicates that these values differed significantly at $p < 0.01$ from the values of the chloretone group. [e] Neutral sterols, cholesterol + coprostanol + coprostanone.

Table 7. Effects of Freshwater Clam Extract (FCE) on Hepatic mRNA Involved Cholesterol Metabolism in Rats Fed a Control or Chloretone-Containing Diet for 2 Weeks[a]

	hepatic mRNA abundance (arbitrary units)[b]						
					ANOVA		
	control group[c]	FCE group[c]	chloretone group[c]	chloretone + FCE group[c]	chloretone	FCE[d]	interaction
HMG-CoA reductase	100 ± 9	86.4 ± 9.9	113 ± 17	144 ± 40	NS[e]	NS	NS
CYP7A1	100 ± 27	263 ± 44	185 ± 30	589 ± 146	0.05	0.01	NS
LXR	100 ± 6	95.8 ± 8.1	108 ± 8	105 ± 9	NS	NS	NS
HNF-4	100 ± 14	101 ± 12	118 ± 7	77.0 ± 6.8	NS	NS	NS
SHP	100 ± 18	117 ± 17	178 ± 30	155 ± 27	0.05	NS	NS
Apo A-I	100 ± 16	51.6 ± 6.5	115 ± 15	78.1 ± 4.7	NS	0.01	NS

[a] Each value is the mean ± SEM for six rats in each dietary group. [b] Hepatic ApoE mRNA level was not affected by any treatment employed in this study, so it was used as a normalization standard. [c] Control group, basal diet; FCE group, FCE-supplemented basal diet; chloretone group, chloretone-supplemented basal diet; chloretone + FCE group, chloretone and FCE-supplemented basal diet. [d] FCE means freshwater clam extract. [e] Statistical significance of differences among values was analyzed by two-way ANOVA. When the interaction (chloretone × FCE) was significant, Student's *t* test was performed. NS, not significant ($p > 0.05$).

DISCUSSION

In previous studies, the hypocholesterolemic effects of freshwater clams were reported in hypercholesterolemic rats fed a high-cholesterol diet (*1*−*3*). The hypocholesterolemic actions of freshwater clam are believed to be mainly due to the interference of cholesterol absorption by phytosterols (*2, 3*). We recently demonstrated that the hypocholesterolemic actions of FCE might also be attributable to the enhancement of cholesterol degradation to bile acids through the induction of CYP7A1 in rats fed a high-cholesterol diet (*1*).

Dietary xenobiotics, such as chloretone, caused increases in serum and hepatic cholesterol levels in rats (*15*−*17*). The hypercholesterolemia induced by xenobiotics is caused by enhancement of hepatic cholesterol synthesis accompanied by the induction of the activity of HMG-CoA reductase (*15, 16*) and its gene expression (*17*). In this study, serum and hepatic cholesterol levels of rats fed a diet supplemented with chloretone as a xenobiotic were elevated in a similar manner as in previous studies (*15*−*17*). However, the expression of hepatic HMG-CoA reductase mRNA was not significantly altered but was slightly up-regulated by supplementation with chloretone (**Table 7**). Although HMG-CoA reductase activity was not measured in this study, the cholesterol level in the serum and liver was elevated by supplementation with chloretone (**Table 4**). Since all rats in this study were fed cholesterol free diets, it was likely that hepatic cholesterogenesis was stimulated by chloretone, and hypercholesterolemia was induced in rats fed a chloretone-containing diet. FCE reduced the serum and hepatic cholesterol levels in normal rats and hypercholesterolemic rats fed a diet supplemented with chloretone, as well as in rats fed a high-cholesterol diet (*1*).

We have indicated in our previous study that FCE might enhance the cholesterol catabolism to bile acids through the induction of CYP7A1 gene expression (*1*). We also suggested the involvement of HNF-4 in the induction of the CYP7A1 gene in rats fed a high-cholesterol diet with FCE (*1*). However, the expression of the HNF-4 gene was not changed, although CYP7A1 gene expression was induced by FCE in rats fed a chloretone-containing diet in this study. We are currently investigating how CYP7A1 is induced by FCE in rats fed a high-cholesterol diet.

The administration of xenobiotics to animals causes hyper-α-lipoproteinemia and an increase in apo A-I (*9, 10*). Oda and Yoshida (*10*) have speculated that feeding xenobiotics might result in an overproduction of apo A-I and HDL. Overproduction of apo A-I was attributed to stimulation of apo A-I gene expression (*27*). Walsh et al. (*28*) indicated that HDL-cholesterol and apo A-I levels were highly correlated in transgenic mice which had the integrated human apo A-I gene. Sorci-Thomas et al. (*29*) have demonstrated a significant positive relationship between the serum apo A-I concentration and the hepatic apo A-I mRNA concentration. In this study, FCE strongly suppressed the expression of the apo A-I gene (**Table 7**). This result suggests the involvement of the suppression of the apo A-I gene expression in the reduction of total and HDL-cholesterol (**Table 4**).

The exposure of xenobiotics induces fatty liver (*11, 12, 18*). Hitomi et al. (*18*) have indicated that fatty liver induced by PCB was attributed to the induction of NADPH-generating enzymes, including malic enzyme (ME, EC 1.1.1.40), glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49), and 6-phosphogluconate dehydrogenase (EC 1.1.1.44) in the liver. In this study, intake of FCE inhibited lipid accumulation in the liver caused by chloretone (**Table 5**). We found in our unpublished study that feeding FCE suppressed the ME and G6PD genes in normal rat liver using DNA microarray techniques. It is likely that these

genes would also be suppressed by FCE in rats fed a chloretone-containing diet.

In conclusion, FCE improves not only exogenous hypercholesterolemia but also xenobiotics-induced hypercholesterolemia, which is characterized by hyper-α-lipoproteinemia. Furthermore, feeding FCE ameliorates fatty liver induced by the exposure to xenobiotics. These actions of FCE might be due to the changes in hepatic gene expression for lipid metabolism such as CYP7A1, apo A-I, ME, and G6PD. However, we still do not know what specific ingredients in FCE are responsible for these beneficial actions, and this will require further investigation in the future.

ABBREVIATIONS

Apo, apolipoprotein; BHT, 2,6-di-*tert*-butyl-*p*-cresol; CYP 7 A1, cholesterol 7α-hydroxylase; DDT, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane; FCE, freshwater clam extract; G6PD, glucose-6-phosphate dehydrogenase; HMG-CoA, 3-hydroxy-3-methyl coenzyme A; HNF-4, hepatocyte nuclear factor 4; LXR, liver X receptor; ME, malic enzyme; PCB, polychlorinated biphenyls; SHP, small heterodimer partner.

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