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## The effect of *Codium fragile* (Chlorophyta) extract on hepatic dysfunction and hyperlipidemia in rats

 Kap Joo Park<sup>3</sup>, Eun Kyoung Hwang<sup>1</sup>, Chan Sun Park<sup>2</sup>, Jae Seok Lee<sup>3</sup>, Myung Hwan Cho<sup>\*3</sup>
<sup>1</sup>Seaweed Research Center, NFRDI, Jeonnam 530-831, (KOREA)

<sup>2</sup>Department of Marine Resources, Mokpo National University, Jeonnam - 534-729, (KOREA)

<sup>3</sup>Department of Biological Sciences, Konkuk University, Seoul - 143-701, (KOREA)

 E-mail : [kkupkj@konkuk.ac.kr](mailto:kkupkj@konkuk.ac.kr)

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### ABSTRACT

To examine the effect of *Codium fragile* on blood cholesterol and lipid metabolism, hyperlipidemia was induced in experimental animal rats through the administration of a hypercholesterolemic diet. *Codium fragile* powder was then administered to the rats for 5 weeks, after which, blood biochemical changes such as blood cholesterol, Aspartate Aminotransferase (AST: serum SGOT) and Alanine Aminotransferase (ALT: serum SGPT) enzyme activity, etc. were determined. And histological changes in liver cells were examined using an electron microscope. *Codium fragile* treatment resulted in a significant reduction of the levels of total cholesterol, blood triglyceride and low-density cholesterol (LDL. Chol) compared to the control rats. In contrast the expression levels of high-density cholesterol (HDL. chol.) were increased. The AST value of the *Codium fragile* administration group was significantly reduced and the blood ALT value of the *Codium fragile* group showed a significant decrease in comparison to the negative control group. Degeneration of hepatic tissues due to the consumption of a high lipid diet for a long period of time was reported in the negative control group; the accumulation of lipid droplets in the cytoplasm was also observed. On the other hand, in the *Codium fragile* administration group, a substantial reduction in the accumulation of lipid droplets in the cytoplasm was seen, and in addition, it was confirmed that the degeneration of hepatic tissues recovered almost to level of that of the normal control group. In summary, this study demonstrated the beneficial possibilities of *Codium fragile* in improving the abnormality of lipid metabolism caused by liver cell damage and hyperlipidemia.

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### KEYWORDS

 Hyperlipidemia;  
*Codium fragile*;  
 Rat liver cell;  
 Lipid metabolism.

### INTRODUCTION

Recently, due to the diversification and westerniza-

tion of dietary habits, the consumption of high calorie food and meats have increased, and consequently, obesity, stroke, arteriosclerosis, hypertension, diabetes, and

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other life style diseases are on the increase. According to the national nutrition survey by the health and welfare department (Korea), in Korean diet, the fat consumption rate per day is continuously increasing from 16.9g in the year of 1969 to 36.8g in 2007<sup>[1]</sup>. Such increase of fat consumption has led to an increase in the incidence and mortality of circulatory diseases by increasing the lipid content in the body.

Among diseases of the circulatory system, hyperlipidemia refers to the condition wherein plasma cholesterol or triglyceride levels are abnormally elevated. In the development of cardiovascular diseases, the concentration of cholesterol has been known to act as an important contributing factor. Blood cholesterol concentration is controlled *in vivo* and maintained constantly. Nonetheless if it were consumed excessively for a long period of time, its blood concentration becomes accumulated and induces hyperlipidemia, arteriosclerosis, coronary artery diseases and other cardiovascular diseases<sup>[15]</sup>.

Chronic high calorie food intake can impair the function of liver cells, which play an important role in the metabolism of nutrients. Hepatic injury can result in severe damage to the serum level of AST, ALT and cholesterol (LDL. Chol and HDL. chol.)<sup>[10-13,16-18]</sup>. The degradation of normal liver functions is indicated by the increased levels of serum AST and ALT enzymes that are normally concentrated in the hepatic tissue. Increased levels of AST and ALT enzymes in the serum can be caused by fatty liver or exposure to by-products, resulting in metabolic problems of the liver and hepatocyte death. And high levels of cholesterol are major causal factors in the development of atherosclerosis and subsequent cardiovascular disease<sup>[8,10,11,17,18]</sup>.

Recently, drugs suppressing 3-hydroxy-3-methylglutaryl CoA reductase (HMG-CoA reductase), which is an enzyme required for the synthesis of cholesterol in hepatocytes, have been reported to be most effective among drugs for the treatment of hypercholesterolemia<sup>[21]</sup>. In addition, cholestyramine, clofibrate, gemfibrozil, nicotinic acid, probucol, etc. have been developed as drugs to decrease blood lipid concentration. However, the effect of these drugs are not constant between various individuals and furthermore, various side effects of these drugs have recently been revealed<sup>[14]</sup>.

Therefore, to resolve the safety problem of the long-term intake of lipid-depression drugs currently supplied by clinics, the appropriate consumption of natural food types has been recommended for the prevention and treatment of cardiovascular diseases<sup>[10-13,16-18]</sup>. Interests in natural diet therapy has heightened as the physiological approach to lowering blood cholesterol level. Studies on the prevention and improvement of hyperlipidemia by applying functional materials extracted from natural food types are also generating widespread interest<sup>[2,7]</sup>.

A type of green algae, *Codium fragile* is used widely as food in Korea, China, Japan as well as Philippines, Hawaii, Africa, and other countries<sup>[3,16]</sup>. It has been used as a helminthic in folk medicine as well as for urinary diseases and obesity treatments<sup>[22]</sup>. The extract of *Codium fragile* contains acrylic acid that has antibiotic activity, anticoagulation activation materials, agglutinin and also anticancer as well as anti-mutation and immune activity<sup>[4,5,14]</sup>. Thus it is a useful marine plant that could be applied in the field of pharmacology and medicine and has the potential to be a candidate material for the treatment of hyperlipidemia as well as obesity<sup>[19,20]</sup>.

This study was therefore conducted to elucidate the effects of *Codium fragile* on hepatic function damaged by hyperlipidemia and elevated blood lipid concentrations in rats. *Codium fragile* extract was administered to rats that were previously maintained on a high-fat diet so as to induce hyperlipidemia. Aspartate Aminotransferase (AST: serum SGOT) and Alanine Aminotransferase (ALT: serum SGPT) activities were measured. Also, the serum major lipid components, i.e. triglycerides, total cholesterol (T.chol.), low-density cholesterol (LDL. chol.), and high-density cholesterol (HDL. chol.) activity were measured. To assess the effects of *Codium fragile* extract on liver cells damaged by hyperlipidemia, a histopathological study on the liver was carried out using electron microscopy.

## MATERIALS AND METHODS

### Experiment animals and diet

As experiment animals, 32 Sprague Dawley male rats, 4 weeks of age, with an average weight of 79.29

TABLE 1 : Composition of basal diet

Ingredients	Contents(%) <sup>a</sup>
Crude protein	22.1
Crude fat	3.5
Fiber	5.0
Crude ash	8.0
Calcium	0.6
Phosphate	0.4

<sup>a</sup>per 200g

TABLE 3 : Experimental designs

Groups	No. of rats	Composition of treatments
Normal control group	8	basic diet + distilled water
Negative control group	8	70% fatty liver inducing feed + 30% basic diet + distilled water
Positive control group	8	70% fatty liver inducing food + 30% basic food + BCPS*
Experiment group	8	70% fatty liver inducing food + 30% Codium fragile powder + distilled water

\*BCPS: Blood circulation promotion solution (mixture of ginkgo biloba extract 120mg and sodium benzoate 60mg/mL, Cho-A Pharmaceutical Co., Ltd., Seoul)

± 1.73g were obtained from Daehan Biolink Co., Ltd. (Seoul). The animals were allowed to adapt to the animal facility for 1 week. The temperature and humidity of the animal room were maintained as 22 ± 2°C and 55 ± 5%, respectively. The rats were kept on the 12 h light/dark cycle and acclimatized to the housing situation. Under a free environment, basal food (Superspeed, Co. Seoul) (TABLE 1), food inducing fatty liver (TABLE 2), and drinking water were freely supplied.

### Classification of experiment groups

After one week of the adaptation period, the rats were divided into 4 groups (n=8) as follows: (i) normal control rats administered with a basic diet + distilled water, (ii) negative control rats administered 70% fatty liver-inducing feed + 30% basic diet + distilled water, (iii) positive control rats administered 70% fatty liver-inducing food + 30% basic food + blood circulation promotion Solution, (iv) experimental control rats administered 70% fatty liver-inducing food + 30% basic food + *Codium fragile* extract (TABLE 3)<sup>[8,18]</sup>. Each feed was prepared by adding 60 g basic diet to 140g fatty liver feed (Dyets INC., New York, USA, DYET# 101865 Custom, AIN-76A Based Purified Rat Diet With 15% Palm Oil, 1.5% Cholesterol, and 0.5% Cholic Acid), and 200g each diet was allowed to be consumed

TABLE 2 : Composition of hyperlipidemic diet

Ingredient	grams/Kg
Casein	200
Sucrose	330
Cornstarch	150
Corn oil	50
Palm oil	150
Cellulose	50

Ingredient	g/Kg
Calcium phosphate dibasic	500.00
Sodium chloride	74.00
Potassium citrate H <sub>2</sub> O	220.00
Potassium sulfate	52.00
Magnesium oxide	24.00
Manganous carbonate	3.50
Mineral mix # 200000	Ferric citrate U.S.P. 6.00
	Zinc carbonate 1.60
	Cupric carbonate 0.30
	Potassium iodate 0.01
	Sodium selenite 0.01
	Chromium potassium sulfate 12H <sub>2</sub> O 0.55
	Sucrose, finely powdered 118.03
	Thiamine HCl 0.60
	Riboflavin 0.60
	Pyridoxine HCl 0.70
	Niacin 3.00
	Calcium pantothenate 1.60
Vitamin mix # 300050	Folic acid 0.20
	Biotin 0.02
	Vitamin B <sub>12</sub> (0.1%) 1.00
	Vitamin A palmitate (500,000 IU/g) 0.80
	Vitamin D <sub>3</sub> (400,000 IU/g) 0.25
	Vitamin E acetate (500 IU/g) 10.00
	Menadione sodium bisulfite 0.08
Sucrose finely powdered	981.15
Cholesterol	15
Choline bitartrate	2
DL-methionine	3
Cholic acid	5

freely for 5 weeks. Distilled water, blood circulation promotion Solution (BCPS) and *Codium fragile* extract was administered orally using a syringe everyday at a constant time.

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TABLE 4 : Body weight gain and ratio of liver weight to body weight administered for 5weeks

Groups	Change of the body weight (g)	Liver (% of the body weight)
	5-0	Mean $\pm$ S.D.
Normal control	307.11 $\pm$ 5.24	5.53 $\pm$ 0.45
Negative control	329.34 $\pm$ 19.58*	13.22 $\pm$ 0.64*
Positive control	321.83 $\pm$ 7.62	12.13 $\pm$ 0.56*
Experimental group	305.39 $\pm$ 0.86* <sup>#</sup>	10.65 $\pm$ 0.79* <sup>#</sup>

Each value was represented as mean $\pm$ standard deviation for 8 rats. Means with different superscripts within a column are significantly different from the normal control at \* $p < 0.05$ , \*\* $p < 0.01$ , and significantly different from negative control at # $p < 0.05$

TABLE 6 : Serum levels of total cholesterol and triglyceride

Groups	Total cholesterol	Triglyceride
	Means $\pm$ S.D	Means $\pm$ S.D
Normal control group	55.25 $\pm$ 10.94	54.75 $\pm$ 10.87
Negative control group	191.50 $\pm$ 63.98*	61.75 $\pm$ 5.19*
Positive control group	130.00 $\pm$ 14.54**	46.25 $\pm$ 6.45*
Experiment treatment group	132.50 $\pm$ 20.34* <sup>#</sup>	55.00 $\pm$ 10.30* <sup>#</sup>

Each value was represented as mean $\pm$ standard deviation for 8 rats. Means with different superscripts within a column are significantly different from the normal control at \* $p < 0.05$ , \*\* $p < 0.01$ , and significantly different from negative control at # $p < 0.05$

Preparation of *Codium fragile* extract and BCPS

Boiling water extracts of *Codium fragile* were prepared from the dried *Codium fragile*. 25g of *Codium fragile* was added into 1,000mL of sterilized water and boiled for 150 min using a herbal and medicinal boiling pot (Daewoong Co., Ltd., Seoul). After centrifugation at 6000  $\times$  g for 15 min, aqueous extracts from the sample were filtered through 3mm filter papers (Whatman, England), and the final volume was adjusted to around 400mL in order to prepare an appropriate volume for administration (about 1.6 g/kg body weight/day)<sup>[10-13,16-18]</sup>. BCPS was mixture of 120mg Ginkgo biloba extract and 60mg/mL sodium benzoate (Cho-A Pharmaceutical Co., Ltd., Seoul), and the dose was calculated by the average weight of experimental animals based on humans (60mg/kg daily) and administered.

## Animal autopsy

On the last day of the experiment, the animals were fasted for 16 hours, the abdomen was resected under

TABLE 5 : Serum levels of AST and ALT

Group	AST	ALT
	Means $\pm$ S.D	Means $\pm$ S.D
Normal control group	131.50 $\pm$ 24.80	42.25 $\pm$ 10.31
Negative control group	951.75 $\pm$ 204.80*	180.00 $\pm$ 17.72*
Positive control group	787.00 $\pm$ 162.45* <sup>#</sup>	154.00 $\pm$ 75.77
Experiment treatment group	662.25 $\pm$ 233.70*	143.50 $\pm$ 61.21 <sup>#</sup>

Each value was represented as mean $\pm$ standard deviation for 8 rats. Means with different superscripts within a column are significantly different from the normal control at \* $p < 0.05$ , \*\* $p < 0.01$ , and significantly different from negative control at # $p < 0.05$

TABLE 7 : Serum levels of HDL-cholesterol and LDL-cholesterol

Groups	HDL	LDL
	Means $\pm$ S.D	Means $\pm$ S.D
Normal control group	24.25 $\pm$ 2.63	9.50 $\pm$ 1.29
Negative control group	13.50 $\pm$ 1.29*	100.25 $\pm$ 27.44*
Positive control group	20.00 $\pm$ 1.83**	55.50 $\pm$ 9.98* <sup>#</sup>
Experiment treatment group	17.50 $\pm$ 3.11* <sup>#</sup>	68.75 $\pm$ 12.53* <sup>#</sup>

Each value was represented as mean $\pm$ standard deviation for 8 rats. Means with different superscripts within a column are significantly different from the normal control at \* $p < 0.05$ , \*\* $p < 0.01$ , and significantly different from negative control at # $p < 0.05$

weakly anesthetized condition with ethyl ether and 3-4mL blood was collected from the venae cavae and distributed into an EDTA-free test tube. Immediately after blood collection, the liver was extracted, washed with cold saline and the weight was measured.

## Biochemical analysis (AST, ALT, HDL-cholesterol, LDL-cholesterol, Total cholesterol, Triglyceride)

The blood collected from each experiment group was kept at room temperature for approximately 30 minutes. The serum was separated by centrifugation (6,000  $\times$  g, 15 min) and used for serum biochemical tests. Aspartate transaminase (AST), a marker enzyme of the deterioration of liver function was measured using an AST (GOT) kit (Bayer Co. Ltd., Frankfurt, German) while alanine transaminase (ALT) was measured using an ALT (GPT) Reagents kit (Bayer Co. Ltd., Frankfurt, German). The total cholesterol concentration was measured using a Cholesterol Reagents kit (Bayer Co. Ltd., Frankfurt, German) by an automated biochemical analyzer (ADVIA 1650/2400, Bayer Co.

Ltd., Frankfurt, German), high-density lipoprotein (HDL) was measured using a Direct HDL-Cholesterol Kit (Bayer Co. Ltd., Frankfurt, German) and low-density lipoprotein (LDL)-cholesterol was measured using a LDL-cholesterol kit (Daiichi Co. Ltd., Tokyo, Japan) by an automated biochemical analyzer (ADVIA 1650, Bayer Co. Ltd., Frankfurt, German). Triglyceride (TG) concentration was measured using a Triglycerides reagents (Bayer Co. Ltd., Frankfurt, German) by an automated biochemical analyzer (ADVIA 1650/2400, Bayer Co. Ltd., Frankfurt, German)<sup>[8,10,11,17,18]</sup>.

### Cytohistochemical analysis

Rats in each of the four groups were anesthetized by inhaling ether and liver tissue (1mm<sup>3</sup>) was removed, washed by administering saline (0.9% NaCl) and fixed in fixative solution (4% paraformaldehyde and 2.5% glutaraldehyde) for 12 hours. The samples were thinly sectioned, fixed with osmium acid (OsO<sub>4</sub>, 2% Osmium tetroxide) for 1 hour at room temperature and washed. The samples were treated with ethanol for 60 minutes at each concentration. The dehydrated tissues were substituted with propylene oxide, embedded in epon resin and polymerized in an incubator (60°C, 72 hours). The polymerized tissues were prepared as 60-80nm thick sections using an ultramicrotome, attached to copper grids (300 mesh), dried, electronically stained with lead citrate in uranyl acetate for 15 minutes and examined by a transmission electron microscope (JEM-2000 EXII, 80KV)<sup>[4,5,10-13,14,16-18]</sup>.

### Statistical analysis

All results were shown as mean ± standard deviation. Statistical evaluation of data was performed by Duncan's multiple range test to make comparisons between groups.

## RESULTS AND DISCUSSION

### Weight gain and ratio of liver weight to body weight

The weight change of experiment animals that were administered with a high fat diet for 5 weeks is shown in TABLE 4. The final weight change of the normal group and the negative control group was shown to be 307.11 ± 5.24g and 329.34 ± 19.58g, respectively. The final weight change of the positive control group was

321.83 ± 7.62g, while the *Codium fragile* administration group was shown to be 305.39 ± 0.86g. It was also found that in the negative control group, the overweight phenomenon was shown in comparison to the normal group. The *Codium fragile* administration group showed a significant weight change similar to the normal group. Thus the effect of the negation of weight gain caused by the high fat diet in the *Codium fragile* administration group was confirmed.

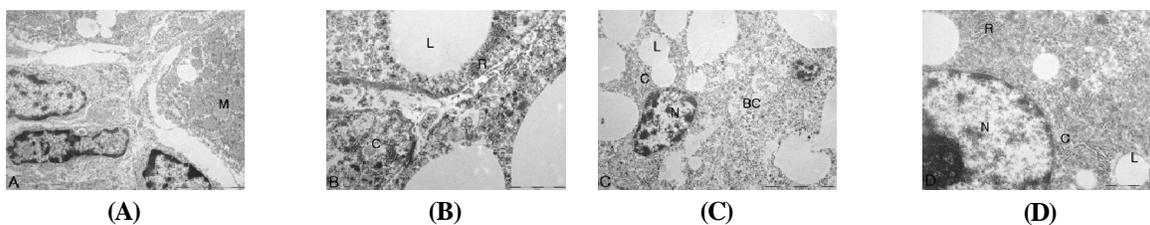
The ratio of total weight of experiment animals and the ratio of body weight to liver weight obtained from our experiment is shown in TABLE 4. After consumption of the high fat diet for a long period, the liver was enlarged because of fatty liver and hepatic fibrosis. Several research results showed that the liver weight of all high fat diet groups was increased by the accumulation of lipid in hepatic tissues due to consumption of a high fat diet<sup>[21]</sup>. In the present study, a similar trend to previously reported studies was shown. The ratio of body weight and the liver of the negative control group was 13.22 ± 0.64%, and it was significantly high in comparison to the normal group of 5.53 ± 0.45%. Thus it was found that fatty liver and liver fibrosis had already progressed; this was in agreement with previous reports showing that the liver became enlarged upon administration of high fat diet for a long period of time.

On the other hand, the positive group was 12.13 ± 0.56%, and a slightly reduced value was shown, and the ratio of body weight and the liver in the experimental group was shown to be 10.65 ± 0.79%, and a significant decreased was shown in comparison with the normal group and the negative control group.

### Activities of AST and ALT

Changes in AST and ALT activity of each group is presented in TABLE 5. AST and ALT levels both increased with increased high fat diet intake. These enzymes are well-documented indicators of hepatic dysfunction, with increased AST and ALT levels reflecting impaired liver function<sup>[14]</sup>. In the normal group, the AST value was shown to be 131.50 ± 24.80 U/L, the value of the negative group showed a rapid increase to 951.75 ± 204.80 U/L, and thus the liver damage caused by the consumption of high fat diet for a long time could be confirmed.

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**Figure 1 :** Changes in the ultrastructure of the hepatocytes of rats. (A) no cytoplasmic changes in the normal control groups. (B) hepatocytes of negative control group show degenerative signs such as clear areas and dilated biliary canaliculi without microvilli and the hepatocytes also exhibit a diffuse accumulation of lipid droplets (L) in the cytoplasm and show the increase of collagen in the hepatic lobule. (C) lipid droplets are slightly increased in the hepatocytes of the positive control group. (D) hepatocytes in experiment group animals shows the normal morphology of hepatocytes (as in the normal control group) with none of the degenerative signs exhibited in the negative control group. M, Mitochondria. N, nucleus of the hepatocyte. C, collagen. R, rough endoplasmic reticulum. BC, bile canaliculus. L, lipid droplet. scale bar = 5 $\mu$ m. Magnification:  $\times 10,000$ .

In the positive group, the AST value was  $787.00 \pm 162.45$  U/L, which showed a slight decrease compared to negative group. The AST value of the *Codium fragile* administration group was  $662.25 \pm 233.70$  U/L. It was measured to be lower than the AST value of the negative control group. The ALT level of the normal group was shown to be  $42.25 \pm 10.31$  U/L, the negative control group was  $180.00 \pm 17.72$  U/L, and it was shown to be markedly higher. This also confirmed the presence of liver damage due to the consumption of the high fat diet for the period of time. The ALT value of the positive control group was shown to be  $154.00 \pm 75.77$  U/L, the ALT value of the *Codium fragile* administration group was shown to be  $143.50 \pm 61.21$  U/L. In this study, the *Codium fragile* administration group exhibited significantly reduced AST and ALT levels compared to the negative control group. These data suggest the possibility of *Codium fragile* being an excellent candidate to ameliorate the effect of hepatocytes and anti-hyperlipidemia from high fat diet-mediated damage in the rat.

### Total cholesterol and triglyceride levels

Serum total cholesterol and triglyceride concentrations are presented in TABLE 6. Total cholesterol concentration of the normal group was  $55.25 \pm 10.94$  mg/dL, while that of the negative control group was increased greatly to  $191.50 \pm 63.98$  mg/dL. Total cholesterol levels in the positive control group and the *Codium fragile* administration groups were shown to be  $130.00 \pm 14.54$  mg/dL and  $132.50 \pm 20.34$  mg/dL, respectively. Serum triglyceride concentration of the normal group, the negative control group, the positive control group, and the *Codium fragile* adminis-

tration group were  $54.75 \pm 10.87$  mg/dL,  $61.75 \pm 5.19$  mg/dL,  $46.20 \pm 6.45$  mg/dL, and  $55.00 \pm 10.30$  mg/dL, respectively. In comparison to the normal control group, the *Codium fragile* administration group showed a significantly low total cholesterol and triglyceride content. Many reports indicated that a high fat diet intake significantly increased both serum and hepatic triglyceride (TG) levels resulting in hypertriglyceridemia and fatty liver<sup>[14]</sup>. Data summarized in TABLE 6 indicates that the administration of *Codium fragile* extract have markedly beneficial effects on the serum lipid levels. Based on reports, indicating that an elevated blood cholesterol level is one of the main causes of vascular disease in the heart and circulatory system<sup>[8,18]</sup>, a number of drugs have been developed to lower plasma cholesterol concentrations, such as cholestyramine, probucol and statins. However, little work has been done in developing natural materials to prevent hyperlipidemia. In this context, this report suggests that *Codium fragile* may represent an alternative therapeutic agent to assist in the prevention and treatment of hyperlipidemia.

### HDL-cholesterol and LDL-cholesterol levels

Serum HDL-cholesterol and LDL-cholesterol concentrations are presented in TABLE 7. HDL-cholesterol has the effect of improving arteriosclerosis and angiosclerosis. LDL-cholesterol accumulates in the artery blood vessel wall primarily and may cause arteriosclerosis, and thus it has been known to be an important risk factor in the development of arteriosclerosis and cardiovascular diseases<sup>[9]</sup>. In our experiments, the HDL-cholesterol level of the normal group was  $24.25 \pm 2.63$  mg/dL, while the negative control group and the

positive control group was shown to be  $13.50 \pm 1.29$  mg/dL and  $20.00 \pm 1.83$  mg/dL, respectively. That of the experiment group was shown to be  $17.50 \pm 3.11$  mg/dL, and in comparison to the normal group, it was significantly low by more than 30%. The LDL-cholesterol of the normal group ( $9.50 \pm 1.29$  mg/dL) and the negative group ( $100.25 \pm 27.44$  mg/dL) was noticeably different. Comparing the positive control group ( $55.50 \pm 9.98$  mg/dL) and the experimental group ( $68.75 \pm 12.53$  mg/dL) with the negative control group, the value of LDL-cholesterol was significantly decreased. It was found that *Codium fragile* directly lowered the blood LDL-cholesterol value and increased HDL-cholesterol value.

In the American NIH standard, for the determination of the risk level of cardiovascular diseases, the arteriosclerosis index and cardiac risk index have been applied<sup>[6]</sup>. In our experiments, the ratio of HDL-cholesterol and total-cholesterol (HTR: HDL-cholesterol/total-cholesterol), the fluctuation of arteriosclerosis index (A.I., atherogenic index: total-cholesterol-HDL-cholesterol / HDL-cholesterol), and cardiac risk factor (C.R.F., cardiac risk factor : total-cholesterol / HDL-cholesterol) was calculated. The HTR of the *Codium fragile* administration group was 2 times higher than that of the negative control group, and thus it was found to reduce the risk of the development of cardiovascular diseases. The arteriosclerosis index of the negative control group showed a value reaching almost 2 times higher than that of the *Codium fragile* administration group. Its cardiac risk index showed a pattern similar to arteriosclerosis index<sup>[6]</sup>.

Hyperlipidemia is an index of arteriosclerosis symptoms. It has been reported to increase the synthesis of triglycerides in the small intestine. The secretion of chylomicron increases the synthesis of triglycerides in the liver, thereby increasing the synthesis and secretion of VLDL-cholesterol and LDL-cholesterol, decrease HDL-cholesterol synthesis and decreasing lipase activity. A decrease in the lipase activity induces the reduction of the removal of triglycerides in peripheral tissues. In our study, similar results were demonstrated in rats wherein an induced dietary hyperlipidemia allowed for the noticeable elevation of the content of total-cholesterol and LDL-cholesterol.

### Cytohistological study

Figure 1 shows the results of the observation of liver cell tissues examined by a transmission electron microscope. Figure 1A is the electron micrograph of the normal group, 1b is the negative control group, 1C is the positive control group, and 1D is the electron micrograph of the *Codium fragile* administration experiment group.

Figure 1(A) depicts the normal morphology of hepatocytes. The mitochondria exhibit a normal morphology. In the electron micrograph of the negative control group, degeneration of liver tissues caused by the consumption of a high fat diet for a long period of time is shown in figure 1(B). Hepatocytes exhibited diffuse accumulation of variously sized lipid droplets. Figure 1c is the result of the positive control group, showing a reduction in fat droplets and degeneration of liver tissues in comparison to the negative control group. And figure 1(C) exhibit moderately dilated biliary canaliculi with short microvilli. 1(D) is the electromicrograph of the *Codium fragile* administration group, and in comparison with the result of the negative control group, a substantial reduction of the accumulation of fat droplets in the cytoplasm was shown. The rough endoplasmic reticulum (R) is well developed and degeneration of liver tissue was decreased to a level similar to that of the normal group.

In summary, a high concentration of total-cholesterol and LDL-cholesterol acts as a major factor in the induction of arteriosclerosis and cardiovascular diseases, and thus, numerous studies and efforts have been made to develop drugs and functional materials. It is anticipated that if drugs or functional materials were developed from natural materials with less side effects, its value would be great. From such a point of view, the results of our study suggest that *Codium fragile* could be tested as a candidate substance for natural therapeutic against hyperlipidemia.

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