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The effect of codium fragile (Chlorophyta) extract on the hepatic dysfunction and hyperlipidemia in rats

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ABSTRACT

To examine the effect of Codium fragile on blood cholesterol and lipid metabolism, hyperlipidemia was induced in the experimental animals rats by administering hypercholesterolemia diet, and while administering Codium fragile powder for 5 weeks, blood biochemical change such as blood cholesterol, AST and ALT enzyme activity, etc. was examined, and histological change of liver cells were examined using an electron microscope. Codium fragile treatment resulted in a significant reduction in the level of total cholesterol, blood triglyceride and LDL-cholesterol compared to the control rats. In contrast, expression levels of HDL-cholesterol 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 with the negative control group. The histological change of liver cells of experimental animals was examined using an electron microscope, and it was found that in the negative control group, degeneration of hepatic tissues due to the consumption of high lipid diet for a long time was shown noticeably, and the accumulation of lipid droplets in the cytoplasm could be observed. On the other hand, in the Codium fragile administration group, a substantial reduction of the accumulation of lipid droplets in the cytoplasm was shown, and in addition, it could be confirmed that the degeneration of hepatic tissues was recovered almost to the normal control group. Summarizing the above results, it showed the possibility of Codium fragile to be a candidate material to improve the abnormality of lipid metabolism caused by liver cell damages and hyperlipidemia.

KEYWORDS

Hyperlipidemia;
Codium fragile;
Rat liver cell;
lipid metabolism.

INTRODUCTION

Recently, due to the diversification and westernization of diet, the consumption of high calorie food and meats is increased, and consequently, obesity, stroke, arteriosclerosis, hypertension, diabetes, and other life style diseases are on the increase. According to the national nutrition survey of the health and welfare department, in Korean diet, the fat consumption rate per day is continuously increasing from 16.9 g in the year of 1969 to 41.6 g in 2001[1]. Such increase of fat consumption becomes a cause of the increase of the inci-

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dence and mortality of circulatory diseases by increasing the lipid content in the body.

Among diseases of the circulatory system, hyperlipidemia refers to the condition that plasma cholesterol or triglyceride is abnormally elevated. In the development of cardiovascular diseases the concentration of cholesterol has been known to act as an important factor, and blood cholesterol concentration is controlled in vivo and maintained constantly. Nonetheless if it were consumed excessively for a long time, its blood concentration becomes high and accumulated in the body and induces hyperlipidemia, arteriosclerosis, coronary artery diseases and other cardiovascular diseases[2].

Recently, drugs suppressing 3-hydroxy-3-methylglutaryl Co A reductase (HMG-CoA reductase) that is an enzyme required for the synthesis of cholesterol in hepatocytes have been reported to be most effective among drugs for hypercholesterolemia[3]. In addition, cholestyramine, clofibrate, gemfivrozil, nicotinic acid, probucol, etc. have been developed as drugs to decrease blood lipid concentration and used, however, the effect is not constant depending on subjects, and problems due to various side effects have been revealed[4].

Therefore, to solve the safety problem of the long term intake of lipid depression drugs currently applied in clinics, recently, the appropriate consumption of natural food types has been recommended for the prevention and treatment of cardiovascular diseases[5]. Interests on the physiological approach method of lowering blood cholesterol level, natural diet therapy is heightened, and the studies on the prevention and improvement effect of hyperlipidemia by applying functional materials extracted from natural food types are required[6,7].

A type of green algae Codium fragile is used widely as food in Korea, China, Japan as well as Philippine, Hawaii, Africa, and others[8,9]. It has been used as a helminthic in folk medicine and used for urinary diseases and obesity treatments[10]. In addition, the extract of Codium fragile contains acrylic acid that has antibiotic activity, anticoagulation activation materials, agglutinin and it also has anticancer as well as anti-mutation and immune activity, etc[11]. Thus it is a useful marine plant that could be applied in the field of pharmacology and medicine and it has the potential to be a candidate material for the treatment of hyperlipidemia as well as obesity treatment[12,13].

Therefore, based on the possibility of Codium fragile as a candidate material for the treatment of hyperlipidemia and obesity treatment, this study was conducted to elucidate the effect of Codium fragile on the hepatic function damaged by hyperlipidemia and blood lipid concentration by experiments. In other words, Codium fragile extract was administered to rats induced hyperlipidemia by maintaining with a high fat method, and Aspartate Aminotransferase (AST: serum SGOT) and Alanine Aminotransferase (ALT: serum SGPT) activity were measured, and the serum major lipid components triglyceride, total cholesterol (T. chol.), low-density cholesterol (LDL. chol.), and high-density cholesterol (HDL. chol.) activity were measured. In addition, to assess the effect of Codium fragile extract on liver cells damaged by hyperlipidemia, the histopathological study on the liver was carried out using an electron microscope.

MATERIALS AND METHODS

Experiment animals and diet

As experiment animals, 32 Sprague Dawley male rats, average weight 79.29 ± 1.73 g and 4 weeks old, were obtained from Daehan Biolink Co., Ltd. (Seoul, Korea). They were adapted to the animal facility for 1 weeks. The temperature and humidity of animal room were maintained as 22 ± 2^0C and 55 ± 5 % , respectively. Rats were kept on the 12 h light/dark cycle and acclimatized to the housing situation. As diet, under a free environment, basal food (Superspeed, Co.) (TABLE 1), food inducing fatty liver (TABLE 2), and drinking water were allowed to consume freely.

Classification of experiment groups

After one week of adaptation period, rats were di-

TABLE 1: Composition of basal diet

Ingredients	Contents(%)[1]
Crude protein	22.1
Crude fat	3.5
Fiber	5.0
Crude ash	8.0
Calcium	0.6
Phosphate	0.4

[1]per 200g

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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**	
Mineral Mix #200000	Calcium phosphate dibasic	500.00	35
	Sodium chloride	74.00	
	Potassium citrate H2O	220.00	
	Potassium sulfate	52.00	
	Magnesium oxide	24.00	
	Manganous carbonate	3.50	
	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 $12H_2O$	0.55	
Vitamin Mix #300050	Sucrose, finely powdered	118.03	10
	Thiamine HCl	0.60	
	Riboflavin	0.60	
	Pyridoxine HCl	0.70	
	Niacin	3.00	
	Calcium pantothenate	1.60	
	Folic acid	0.20	
	Biotin	0.02	
	Vitamin B12 (0.1%)	1.00	
	Vitamin A palmitate (500,000 IU/g)	0.80	
	Vitamin D3 (400,000 IU/g)	0.25	
	Vitamin E scetate (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	

vided to 4 groups (n=8) as follows: (π) normal control rats administered basic diet + distilled water, (θ) negative control rats administered 70 % fatty liver inducing feed + 30 % basic diet + distilled water, (ρ) positive control rats administered 70 % fatty liver inducing food + 30 % basic food + Blood Circulation Promotion Solution, (σ) experimental control rats administered 70 % fatty liver inducing food + 30 % basic food + Codium fragile extract[14]. (TABLE 3). Each feed was prepared by adding 60 g basic diet to 140 g fatty liver feed (Dyets INC., DYET# 101865 Custom, AIN-76A Based Purfied Rat Diet With 15% Palm Oil, 1.5% Cholesterol, and 0.5% Cholic Acid), and 200 g each diet was

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 benxoate 60mg/ml, Cho-A Pharmaceutical Co., Ltd., Seoul)

allowed to consume 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.

Preparation of codium fragile extract and BCPS

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

Animal autopsy

The last day of experiment, animals were fasted for 16 hours, the abdomen was resected under the weakly anesthetized condition with ethyl ether and 3-4 ml blood was collected from the venae cavae and distributed to a 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

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TABLE 4: Body weight gain and ratio of liver weight to body weight

Groups	Change of the body weight(g) after 5 weeks treatment 5-0	Liver(% of the body weight) Mean ± S.D.
Normal control	307.11 ± 5.24	5.53 ± 0.45
Negative control	329.34 ± 19.58*	13.22 ± 0.64*
Positive control	321.83 ± 7.62	12.13 ± 0.56*
Experimental groupup	305.39 ± 0.86*	10.65 ± 0.79*

Each value was represented as mean±standard deviation for 8 rats. Statistical evaluation of data was performed by Wilcoxon rank sum test to compare between groups * p<0.05, ** p<0.01.

minutes. The serum was separated by centrifugation (6,000×g, 15 min) and used for serum biochemical test. In regard to analysis categories, aspartate transaminase (AST) that is a marker enzyme of the deterioration of liver function was measured using a AST (GOT) kit (Bayer, USA) and alanine transaminase (ALT) was measured using a ALT (GPT) Reagents kit (Bayer, USA). In addition, total cholesterol concentration was measured using a Cholesterol Reagents kit (Bayer, USA) by an automated biochemical analyzer (ADVIA 1650/ 2400, Bayer, Japan), high-density lipoprotein (HDL) was measured using a Direct HDL-Cholesterol Kit (Bayer, USA) and low-density lipoprotein (LDL)-cholesterol was measured using a LDL-cholesterol kit (Daiichi, Tokyo Japan) by an automated biochemical analyzer (ADVIA 1650, Bayer, Japan) and Triglyceride (TG) concentration was measured using a Triglycerides reagents (Bayer) by an automated biochemical analyzer (ADVIA 1650/2400, Bayer, Japan)[15,16].

Cytohistochemical analysis

Rats of each four groups were anesthetized by inhaling ether and liver tissue (1mm) 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 thin sectioned, fixed with osmium acid (OsO4, 2 % Osmuim 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 propyrene oxide, embedded in epon resin and polymerized in an incubator (60^0C, 72 hours). The polymerized tissues were prepared as 60-80 nm thick sections using an ultramicrotome, attached to copper grids (300 mesh), dried, electronic stained with lead citrate in uranyl acetate for 15 minutes and examined by a transmission electron microscope (JEM-2000 EXII, 80KV)[17,18].

Statistical analysis

All results are 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 administered high fat diet for 5 weeks was shown in TABLE 4. In our experiment, the final amount of the weight change of the normal group and the negative control group was shown to be 307.11 ± 5.24 g and 329.34±19.58 g, respectively. The final amount of the weight change of the positive control group was 321.83 ± 7.62 g, on the other hand, the Codium fragile administration group was shown to be 305.39 ± 0.86 g. And it was found that in the negative control group, overweight phenomenon was shown in comparison with 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 in our experiment is shown in TABLE 4. Consumed high fat diet for a long time, the liver is enlarged because of fatty liver and hepatic fibrosis. Several research results that the liver weight of all high fat diet groups was increased by the accumulation of lipid in hepatic tissues due to high fat diet have been reported[3]. In our study, a trend similar 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 with the normal group of 5.53 ± 0.45 %. Thus it was found that the fatty liver and liver fibrosis were progressed, it could be found that it is in agreement with the above previous reports showing that administered high fat diet for a long, the liver became

TABLE 5: Serum levels of AST and ALT

Groups	AST	ALT
	Means ± S.D	Means ± S.D
Normal control group	131.50 ± 24.80	42.25 ± 10.31
Negative control group	951.75±204.80*	180.00 ± 17.72*
Positive control group	787.00±162.45*	154.00 ± 75.77
Experiment treatment group	662.25±233.70*	143.50 ± 61.21

Each value was represented as mean±standard deviation for 8 rats. Statistical evaluation of data was performed by Wilcoxon rank sum test to compare between groups *p<0.05, **p<0.01

TABLE 6: Serum levels of total cholesterol and triglyceride

Groups	Total cholesterol	Triglyceride
	Means ± S.D	Means ± S.D
Normal control group	55.25 ± 10.94	54.75 ± 10.87
Negative control group	191.50 ± 63.98*	61.75 ± 5.19*
Positive control group	130.00 ± 14.54**	46.25 ± 6.45*
Experiment treatment group	132.50 ± 20.34*	55.00 ± 10.30**

Each value was represented as mean±standard deviation for 8 rats. Statistical evaluation of data was performed by Wilcoxon rank sum test to compare between groups * p<0.05, ** p<0.01.

enlarged.

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 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

The amount of the change of AST and ALT activity of each group was presented in TABLE 5. AST and ALT levels both increased with increased high fat diet intake. These enzymes are well-documented indicators of hepatic disfunction, with increased AST and ALT levels reflecting impaired liver function. 4) In our study, in the normal group, 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. In the positive group, AST value was 787.00±162.45 U/L, which showed a slight decreased. AST value of the Codium fragile administration group was 662.25 ± 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±10.31 U/L, the negative control group was 180.00±17.72 U/L, and it was shown to be markedly higher. This also confirms the liver damage by the consumption of high fat diet for a long time. The ALT value of the positive control group was shown to be 154.00±75.77 U/L, the ALT value of the Codium fragile administration group was shown to be 143.50±61.21 U/L. In this study, the Codium fragile administration group exhibited significantly reduced AST and ALT levels compared with 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 concentration are presented in TABLE 6. Total cholesterol concentration of the normal group was 55.25 ± 10.94mg/dl, on the other hand, the negative control group was increased greatly to 191.50 ± 63.98 mg/dl, and the positive control group and the Codium fragile administration group were shown to be 130.00 ± 14.54 mg/dl and 132.50 ± 20.34 mg/dl, respectively. Serum triglyceride concentration of the normal group, the negative control group, the positive control group, and the Codium fragile administration group was 54.75 ± 10.87 mg/dl, 61.75 ± 5.19 mg/dl, 46.20 ± 6.45 mg/dl, and 55.00 ± 10.30 mg/dl, respectively. In comparison with the normal control group, the Codium fragile administration group showed a significantly low total cholesterol and triglyceride content. Many reports indicate that high fat diet intake significantly increases both serum and hepatic triglyceride (TG) levels resulting in hypertriglyceridemia and fatty liver[4]. Data summarized in TABLE 6 indicates that the administration of Codium fragile extract have markedly beneficial effects upon serum lipid levels. Based on reports[14]. Indicating that an elevated blood cholesterol level is one of the main causes of vascular disease in the heart and circulatory system, 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 fragilel may represent an alternative therapeutic agent to assist in the prevention and treatment of hyperlipidemia

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HDL-cholesterol and LDL-cholesterol levels

Serum HDL-cholesterol and LDL-cholesterol concentration are presented in TABLE 7. HDL-cholesterol has the effect of improving arteriosclerosis and angiosclerosis, and LDL-cholesterol is accumulated on the artery blood vessel wall primarily and may cause arteriosclerosis, and thus it has been known to be an important risk factor of the development of arteriorsclerosis and cardiovascular diseases[19]. In our experiments, examining the change of HDL-cholesterol and LDL-cholesterol, HDL-cholesterol of the normal group was 24.25 ± 2.63 mg/dL, the negative control group and the positive control group was shown to be 13.50 ± 1.29 mg/dL and 20.00 ± 1.83 mg/dL, respectively, the experiment group was shown to be 17.50 ± 3.11 mg/dL, and in comparison with the normal group, it was significantly low by more than 30 %. It could be found that LDL-cholesterol of the normal group (9.50± 1.29 mg/dL) and the negative group (100.25 ± 27.44 mg/dL) was noticeably different. Comparing the positive control group (55.50 ± 9.98 mg/dL) and the experiment group (68.75± 12.53 mg/dL) with the negative control group, the value of LDL-cholesterol was significantly decreased. It was found that Codium fragile has an effect on the vascular diseases by directly lowering blood LDL-cholesterol value and increasing HDL-cholesterol value.

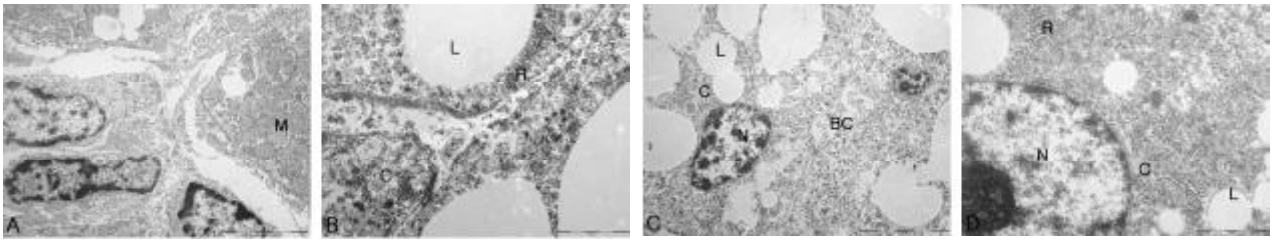
TABLE 7: Serum levels of HDL-cholesterol and LDL-cholesterol

Groups	HDL	LDL
	Means ± S.D	Means ± S.D
Normal control group	24.25 ± 2.63	9.50 ± 1.29
Negative control group	13.50 ± 1.29*	100.25 ± 27.44*
Positive control group	20.00 ± 1.83**	55.50 ± 9.98*
Experiment treatment group	17.50 ± 3.11*	68.75 ± 12.53*

Each value was represented as mean±standard deviation for 8 rats. Statistical evaluation of data was performed by Wilcoxon rank sum test to compare between groups * p<0.05, ** p<0.01

In the American NIH standard, for the determination of the risk level of cardiovascular diseases, arteriosclerosis index and cardiac risk index have been applied. 20) In our experiment, 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. HTR of the Codium fragile administration group was 2 times higher than 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 the Codium fragile administration group. And its cardiac risk index showed a pattern similar to arteriosclerosis index[20].

Hyperlipidemia is an index of arteriosclerosis symptoms. It has been reported to increase the synthesis of triglyceride in the small intestine and the secretion of chylomicron, increase the synthesis of triglyceride in the liver, increase the synthesis and secretion of VLDL-cholesterol and LDL-cholesterol, decrease HDL-cholesterol synthesis and decrease lipase activity, and thus induce the reduction of the removal of triglyceride in peripheral tissues. In our study, similar results were shown. It was detected that in rats induced dietary hy-



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 shows 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 = 5μm. Magnification: ×10,000.

Figure 1: Changes in the ultrastructure of the hepatocytes of rats

perlipidemia, the content of total-cholesterol and LDL-cholesterol was elevated noticeably.

Cytohistological study

Figure 1 shows the result 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 the 1D is the electron micrograph of the Codium fragile administration experiment group.

In the electron micrograph of the negative control group, degeneration of liver tissues caused by the comsumption of high fat diet for a long time was shown, in addition, the accumulation of fat droplets in the cytoplasm due to it could be observed. 1C is the result of the positive control group, and it shows that fat droplets and the degeneration of liver tissues were reduced in comparison with the negative control group. 1D is the electrom micrograph 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, and, in addition, a significant result that the degeneration of liver tissue was decreased to the level similar to the normal group was shown.

As a result, high concentration of total-cholesterol and LDL-cholesterol acts as a major factor of inducing argeriosclerosis and cardiovascular diseases, and thus, numerous studies and efforts have been made to develop drugs and functional materials. It is anticipated if drugs or functional materials were developed from natural materials with less side effects, its value would be great. From such point of view, it is considered that the result of our study suggests the possibility of Codium fragile as a candidate substance for a natural therapeutic for hyperlipidemia.

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