A THESIS
FOR THE DEGREE OF MASTER OF SCIENCE

Effects of *Ecklonia cava* on Plasma and Liver Lipids, Platelet Aggregation and RBC Membrane Stability in Rats Fed Cholesterol Diet

Wan-Ting Yeh

DEPARTMENT OF FOOD SCIENCE AND NUTRITION

GRADUATE SCHOOL
JEJU NATIONAL UNIVERSITY

2010. 2.

Effects of *Ecklonia cava* on Plasma and Liver Lipids, Platelet Aggregation and RBC Membrane Stability in Rats Fed Cholesterol Diet

指導敎授 姜 晶 淑

葉 宛 婷

이 論文을 理學 碩士學位 論文으로 提出함

2010 年 2 月

葉宛婷의 理學 碩士學位 論文을 認准함

審査委員長 _____ ㊞

委 員 _____ ㊞

委 員 _____ ㊞

濟州大學校 大學院

2010 年 2 月

CONTENTS

ABSTRACT

Effects of *Ecklonia cava* on Plasma and Liver Lipids, Platelet Aggregation and RBC Membrane Stability in Rats Fed Cholesterol Diet

Wan Ting Yeh
Department of Food Science and Nutrition, Graduate School
Jeju National University, Jeju, Korea

We compared bioactivity of 10% *Ecklonia cava* (EC) powder, 5% extract (carbohydrase hydrolyzed product) and 3% Dieckol (EC polyphenol) in aspect of antiobese, hypolipidemic, antiplatelet and antioxidant on RBC membrane and TBARS production in rats fed cholesterol diets.

The final weight with low food efficiency ratio in rats fed Dieckol was significantly decreased compared with the control ($p<0.05$). Plasma total cholesterol was significantly decreased in EC powder ($p<0.05$) and HDL-cholesterol was significantly decreased in Dieckol ($p<0.05$) both compared with the control. Liver total cholesterol significantly decreased in EC powder compared with EC extract ($p<0.05$) and liver TG was significantly decreased in Dieckol compared with control ($p<0.05$). Platelet aggregation both in initial slope and the maximum was significantly decreased in Dieckol compared with the control (both, $p<0.05$). Hemolysis both with and without AAPH treatment was significantly low in EC extract compared with other groups ($p<0.05$), but erythrocyte Na leak increase after AAPH treatment was significantly high in EC extract compared with other groups ($p<0.05$). Platelet rich plasma (PRP) and liver TBARS productions were decreased in all EC groups, and PRP TBARS was significantly decreased in Dieckol compared with the control. GOT compared with the control and GPT compared with Dieckol were significantly decreased in rats fed EC powder ($p<0.05$).

In conclusion, polyphenol rich Dieckol from *Ecklonia cava* seems to have some favorable effects in weight gain, platelet aggregation, RBC membrane stability and lipid peroxidation, but it is not clear how it acts at this point.

LIST OF TABLE

List of Figures

Ⅰ. INTRODUCTION

Marine plants such as brown and green algae are of high interest as natural food source that have protective roles in the pathogenesis of various degenerative diseases. Numerous studies have focused on multifunctional biological activities of seaweed and the possibility of their application to functional food stuffs and medicinal purposes. Marine macroalgae are a rich source of various natural antioxidants such as catechin, flavonols, flavonol glycosides which play an important role in preventing lipid peroxidantion and the related diseases such as cancers, inflammatory and cardiovascular diseases (Heinrich *et.,* 2008).

Phlorotannins, identified from several brown algal families are a group of oligomeric polyphenol of phloroglucinol unit and the only phenolic group detected in brown algae. Phlrotannins such as eckol, dieckol, phlorofucofuroeckol were identified in Ecklonia species and responsible for the biological activities of *Ecklonia cava* (EC). Recently, an increasing amount of evidence has demonstrated that *Ecklonia cava* polyphenol exhibits radical scavenging activity, matrix metalloproteinase inhibitory activity, bactericidal activity, protease inhibitory activity, antioxidative activity, and anti-inflammatory (Jimenez-Escrig *et al.,* 2000).

Fucoidan, sulfate ester containing polysaccharide in brown algae also has been shown bioactivities in many researches (Al-Shorepy *et al.,* 2001). Mucus material of fucoidan is characterized as soluble fiber in human, but it has been issued on its bioavailability and bioactivity which depends on the processing that determine the molecular weight of fucus-sulfate ester compound (Bo Li *et al.*, 2008). Fucoidan is structually similar to heparin and have shown various biological activities including anticoagulant, antithrombotic, hypolipidemic and also antioxidant

effects. Dietary intervention with hypocholesterolemic agents is one of the effective methods to reduce serum cholesterol levels. Seaweeds such as Undaria pinnatifida, Laminaria digitata and Ecklonia stolonifera had been proved to be antihyperlipidemic in the pass (Kang *et al*. 2001; Yoon *et al*. 2008).

Ecklonia cava (EC), a brown algae belongs to the Laminariaceae family and abundant in the subtidal coastal regions of Jeju Island in Korea. Korean people have a long tradition of consuming seaweeds such as *U pinatifida*, *L laminaria* and *E stolonifera* as part of their diets. Unlike those favorite seaweeds, *Ecklonia cava* is not yet generally accepted as food stuff, but its polyphenol rich extracts such as eckol, dieckol, phlorofucofuroeckol and fucoidan are actively ongoing hot subjects for researches.

We chose three dietary forms of E. cava including intact EC powder, carbohydrase hydrolyzed extract and dieckol, and examined the antioxidant and antihyperlipidemic effects of EC polyphenol and EC polysaccharide in order to provide data to support future clinical trials. According to SJ Heo (2003), EC powder, EC extract and Dieckol contained carbohydrate of 70%, 40%, 16% and polyphenol of 10−20%, 20%, 46% respectively. The experiment was conducted to compare hypocholesterolemic and antioxidant effects of 10% *Ecklonia cava* (EC) powder, 5% extract and 3% Dieckol by measuring plasma and liver lipids, erythrocyte Na leak, platelet aggregation and TBARS production in rats fed cholesterol diets.

Ⅱ. LITERATURE REVIEW

1. Antioxidant and physiological functions

Numerous epidemiological studies suggest that diets rich in phytochemicals and antioxidant execute a protective role in health and disease. Frequent consumption of fruits and vegetables is associated with a lowered risk of cancer, heart disease, hypertension and stroke. Physiological functions of plant antioxidant are based on its radical scavenging activity preventing oxidative modification on biomembrane, lipoprotein, platelet and prostanoid (Terao *et al.,* 1994; Salah *et al.*, 1995). Consumption of catechin and quercetin or drinking grape juice inhibited platelet aggregation in human (Pignatelli *et al.*, 2000; Keevil *et al.,* 2000). Platelet aggregation and its release reaction were affected by the condition of oxidative stress, and platelet superoxide and TXA2 generation were reduced by plant antioxidant (Ryszawa *et al.*, 2006). Hubbard *et al.*(2006) showed that antioxidant quercetin in onion is involved in tyrosine phosphorylation in signaling pathway causing a reduced platelet aggregation. Antiplatelet compounds such as herb extracts can suppress platelet activity *in vitro* at high concentration, but at low concentration those can stimulate platelet aggregation (Chew *et al.,* 2001).

Murakai *et al.* (2005) proposed that The major tea catechin ,EGCG paticipate regeneration of alpha-tocopherol and ascorbate in the plasma membrane and prevents lipid peroxidation. The extent of oxidative membrane damage has been measured by hemolysis test (Draper *et al,,* 1969) or erythrocyte Na leak which can be increased upon exposure to oxygen generating system (Maridonneau *et al.,* 1983). Erythrocyte Na leak is Na efflux through lipid bilayer of cell membrane in manner of passive diffusion by concentration gradient. Upon exposure to AAPH

radical generating system, Na leak would increase with the extent of membrane damage. Plant flavonoid quercetin, fisetin and morin inhibited lipid peroxidation and enhance RBC membrane integrity against hypotonic lysis (Chaudhuri *et al.,* 2007). Yang *et al.* (2006) reported that Taiwanese herbal medicine Bidens pilosa extracts protect normal human erythrocyte against oxidant damage *in vitro*, and prevented the decline of superoxide dismutase (SOD) activity and the depletion of cytosolic glutathione (GSH) and ATP in erythrocytes.

TBARS is a product of membrane lipid proxidation, and TBARS production will be correlated with leak increase. Diet supplememted coenzyme was effective in reducing TBARS production in PRP (Kim *et al.,* 2007). Coothan *et al.* (2006) reported that there was a positive results on decreasing plasma TBARS levels by fed fucoidan diet. G. *et al.* (2007) also showed that lipid peroxidation was much lower in seaweed treated animal.

Wong *et al.* (2000) showed that fed seaweed diet reduced the CCl_4-induced acute elevation in the levels of GPT and GOT in rats. Jerzy *et al.* (2008) also reported that supplementation of a diet with green tea extract had declined liver GOT and GPT levels in diabetic rats. In addition, Shinhi *et al.* (2007) found that fucoidan reduced CCl_4- induced acute and chronic liver failure. Manal *et al.* (2008) showed that green tea polyphenols can reduce GOT and GPT of plasma.

2. Plant fiber and physiological roles

Ecklonia cava dominating in Jeju Island contains physiologically active polysaccharide, fucoidan. In addition to neutral polysaccharides, such as cellulose and laminarin, and the well-known polyanionic polysaccharide alginic acid, they contain fucoidans which are highly sulfated polysaccharides also can be dietary soluble fiber (Wang *et al.,* 2008).

Dietary soluble fiber intake has been inversely related to the risk of developing coronary heart disease. Consumption of dietary soluble fiber leads to lowering of plasma LDL cholesterol. The intestinal lumen has been widely accepted as the primary site of action of fiber. One of the major primary mechanisms suggests to explain the fiber-mediated lowering of plasma LDL cholesterol is the interruption of the enterohepatic circulation of bile acids, which alters hepatic cholesterol homeostasis (Suheeta *et al.,* 2002). Hara *et al.* (1999) suggested that short chain fatty acid (SCFA) fermented from dietary fibers might be effective in reducing plasma total cholesterol. Short chain fatty acids produced by microflora in large intestine suppress cholesterol synthesis in rat liver and intestine, and SCFA produced from sugar beet fiber decreased plasma cholesterol by interrupting enterohepatic bile circulation (Hara *et al.*, 1998). Kang *et al.* (2001) reported that diet containing seaweed reduced cholesterol absorption and increased fecal sterol excretion. Yoon *et al.* (2008) found that Dieckol from Ecklonia stolonifera decreased in the serum triglyceride, total cholesterol and LDL-cholesterol levels of hyperlipidemic rats. Huang *et al.* (2005) also reported that fed seaweed polysaccharide diet can decrease the blood glucose and the triglyceride, total cholesterol in serum and increase HDL-cholesterol in diabetic rats. In addition, Wong *et al.* (1999) showed that seaweed Hypnea charoides from Hong Kong increased the HDL-cholesterol and decreased the triglyceride and LDL-cholesterol in the serum of rats. Yu *et al.* (2003) reported that fed Ulvan-based diet in rats from green seaweed Chlorophyta was lowered the levels of serum total cholesterol and LDL-cholesterol. Besides plant fiber in lowering cholesterol, plant polyphenol affect cholesterol metabolism. Soboloya *et al.* (2006) reported plant polyphenol fraction interrupted cholesterol absorption and reduced plasma cholesterol.

The effectiveness of seaweed in increasing faecal cholesterol

excretion is attributed to the fact they are acidic polysaccharides which produce an indigestible ionic colloid. (Jimenez *et al.,* 2000)

Dietary red wine had favorable effects on human plasma high-density lipoprotein and blood chemistry (Levy *et al.,* 1994). There are also reports that tea derived catechin had hypocholesterolemic and antihypertensive effects or tea flavonoids retarded LDL oxidation (Arai *et al.,* 2000). Based on previous researches, we assume that *Ecklonia cava* would have prophylactic and therapeutic effects for human health.

Ⅱ. MATERIALS & METHOD

1. Animal and diets

Forty of eight weeks old Sprague Dawley rats (Orient Bio Co, Ltd, Gapyung, Korea) were divided into four groups and fed the following experiment diets: 0.5% cholesterol based control diet; control diet plus 10% EC powder; control diet plus 5% EC-extract and control diet plus 3% Dieckol (Table 1). Rats had free access to water and were housed individual cages in a room maintained at 20-25℃ with 12 hour dark-light cycle. Rats had an libitum access to their respective diets and water for 4 weeks. Food intake for individual rats was monitored every 2 days and animals were weighted every 2 days during the feeding period.

Ecklonia cava of powder, extract and dieckol were kindly provided by Dr. Chun Yu-Jin in College of Ocean Science, Jeju National University. *Ecklonia cava* was collected along the coast of Jeju island during the season of May, 2007. *Ecklonia cava* after removed salt, sand and epiphytes using tap water was dried in Far Infrared Dryer (JOURI-Q, KEC, Korea) and powdered. *Ecklonia cava* extract was prepared by a hydrolyzing. *Ecklonia cava* with carbohydrase, and Dieckol was prepared by fractionation process of polyphenol rich part of *Ecklonia cava* using HPLC. Carbohydrate proportion of powder, extract and dieckol was 70%, 40.02% and 15.69%, and the proportion of polyphenol in powder, extract and dieckol was 10.2%, 20.09% and 45.99%.

Table 1. Composition of experimental diets (%)

Ingredient	Control	*Ecklonia cava* powder	*Ecklonia cava* extract	Dieckol
Casein[a)]	20.0	20.0	20.0	20.0
L-methionine[a)]	0.3	0.3	0.3	0.3
Lard	9.0	9.0	9.0	9.0
Soybean Oil	1.0	1.0	1.0	1.0
Choline chloride[b)]	0.2	0.2	0.2	0.2
Vitamin mix[c)]	1.0	1.0	1.0	1.0
Mineral mix[d)]	3.5	3.5	3.5	3.5
Sucrose	20.0	20.0	20.0	20.0
Corn starch	39.3	34.3	36.3	36.3
Cellulose	5.0	-	3.0	5.0
Cholesterol[e)]	0.5	0.5	0.5	0.5
Cholic acid[e)]	0.2	0.2	0.2	0.2
EC powder[1)]		10.0		
EC extract[2)]			5.0	
Dieckol[3)]				3.0
Total (%)	100.0	100.0	100.0	100.0

a) Teklad, Harlan Madison WI, USA

b) Junsei Chemical Co., Ltd.

c) Vitamin mixture(mg/100g) :Thiamine HCl 60.0, Riboflavin 60.0, Pyridoxine HCl 70.0, Nicotinic Acid 300.0, D-Calcium Pantothenate 160.0, Folic Acid 20.0, D-Biotin 2.0, Vit. B_{12} 0.1, Vit. A 80.0, Vit. E 2000.0, Vit. D_3 0.25, Vit. K 0.5, Sucrose 97290.0

d) Mineral mixtuer(g/100g) : $CaHPO_4$ 50.0, NaCl 7.4, $K_3C_6H_5O_7 \cdot H_2O$ 22.0, K_2SO_4 5.2, MgO 2.4, Manganous carbonate(43-48%Mn) 0.35, Ferric citrate(16.7%Fe) 0.6, Zinc carbonate(70% Zn) 0.16, Cupric carbonate(53-55%Cu) 0.03, KIO_3 0.001, $Na_2SeO_3 \cdot 5H_2O$ 0.001, $CrK(SO_4)_2 \cdot 12H_2O$ 0.055, Sucrose 11.804

e) Sigma Chemical Co., USA

1) *Ecklonia cava* powder after Far Infrared radiation drying

2) Hydrolyzed extract using carbohydrase

3) Polyphenol rich extract of *Ecklonia cava*

2. Analysis of sample

At the end of 4 weeks, rats were anesthetized with ether after fasted for 12 hours, and blood was collected by cardiac puncture into vacuum tubes containing heparin. Plasma was obtained by centrifugation of blood sample at 2000×g and stored at −20℃ for later analysis. Hematocrit of the whole blood was determined using Hematocrit centrifuge. The liver was quickly removed and weighted and frozen at −20℃ until analyzed.

1) Whole blood platelet aggregation

Platelet aggregation was measured using Aggrolink attached Chronolog whole blood aggregometer (model 500 Ca Havertown, Pennsylvania, USA). The whole blood was diluted with isotonic saline (1:4) to give a platelet concentration of approximately 400,000/ul. Two uM adenosine diphosphate (ADP) was added to initiate aggregation, and three readings of impedance(Ω) changes were taken for each rat and the mean value was used. Platelet aggregation causes an increased in impedance across two platinum electrodes in whole blood and the impedance gain was set 20Ω in recorder response. The impedance method using the fresh whole blood has the advantage of measuring platelet aggregation under nearly physiological conditions in the presence of other blood components.

2) Plasma HDL-cholesterol, Total-cholesterol and Triglyceride

Total cholesterol and glucose and triglyceride were measured using commercial enzymatic assay kit (ASAN Pharmaceutical Co., Ltd, Korea). For total cholesterol, HDL-cholesterol, triglyceride and glucose assay, 20ul each of plasma sample was used for quanitiation, and the absorbance for total cholesterol, HDL-cholesterol and glucose was read

at 500nm and triglyceride at 550nm using spectrophotometer (Uvikon XS, Secomam Co., France)

3) Liver Total cholesterol & Triglyceride

Liver samples were prepared by modifying the method described in Folch *et al.* (1956) to determine cholesterol and triglyceride. One gram of liver tissue was homogenized in 6ml chloroform/methanol mixture (2/1, v/v) and 2ml distilled water using a tissue homogenizer for 5 minutes and centrifuged at 1000×g for 10 minutes. The chloroform fraction containing cholesterol and triglyceride is the bottom layer. For liver total cholesterol, five hundreds ul of chloroform fraction in the bottom layer was transferred and leaved to dry for 24 hours. Fifty ul Triton X-100:chloroform (1:1,v/v) was added and vortexed, and 450ul chloroform was added to be 500ul and vortexed again. Ten ul was transferred to a new tube and leaved to dry under clean bench, then 1.5ml color regent (ASAN Pharmaceutical Co., Ltd, Korea) was added and incubated at 37℃ water bath for 5 minutes. The absorbance of the incubation medium was read at 500nm using spectrophotometer.

For liver triglyceride, Ten ul of chloroform fraction in bottom layer was transferred to a new tube and leaved to dry under clean beach, then 50ul methanol was added and vortexed. To this solution, 1.5ml color regent (ASAN Pharmaceutical Co., Ltd, Korea) was added and incubated at 37℃ water bath for 10 minutes. The absorbance of the incubation medium was read at 500nm using spectrophotometer.

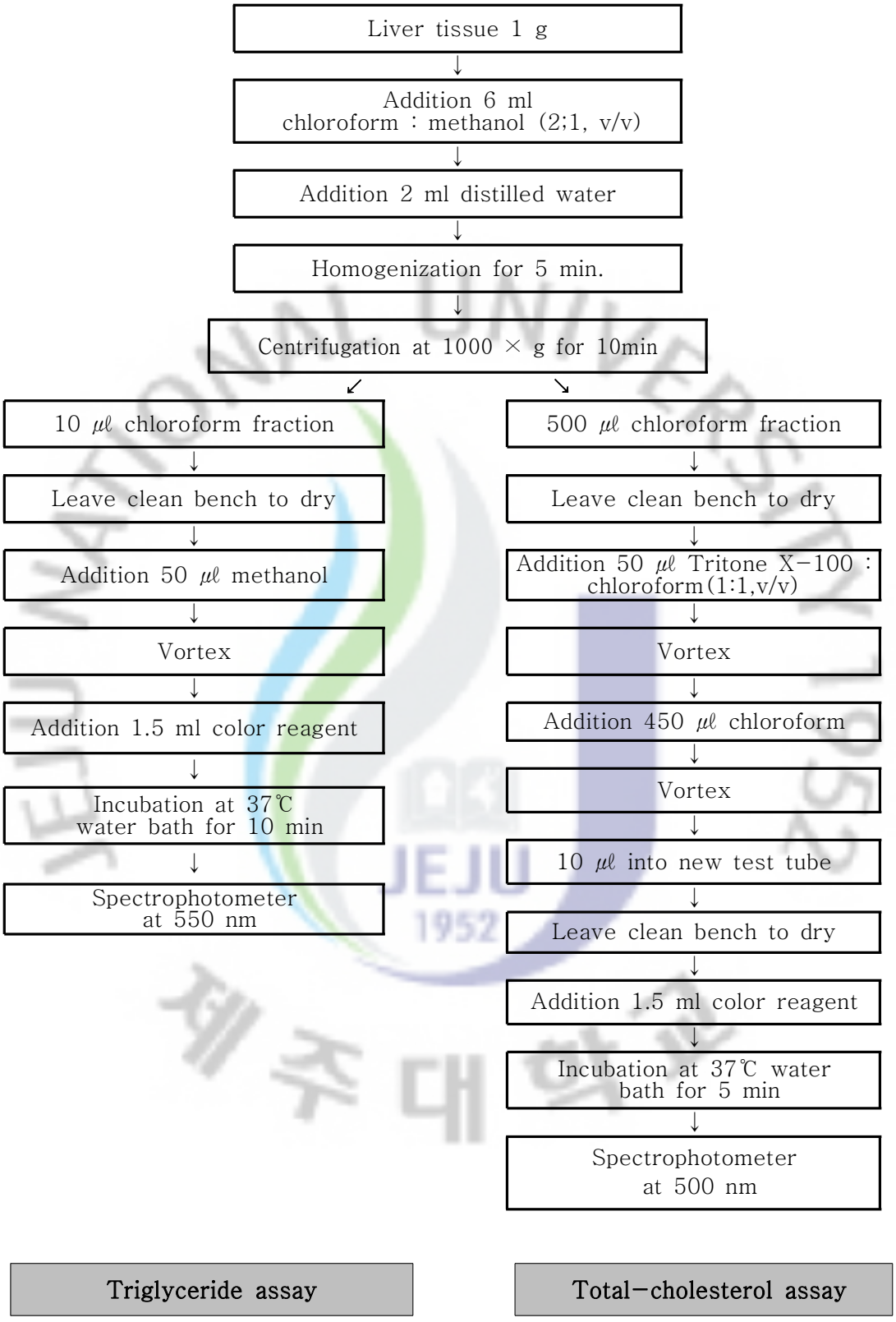

Figure 1. Analytical Scheme for Extraction of Liver Lipids

4) Erythrocyte Na-leak

Red cell preparation:

Blood was centrifuged at 1000×g for 10 minutes, and the plasma and buffy coat were removed. Red blood cells were washed 5 times with cold isotonic washing solution [150mM choline chloride, 10mM Tris-4 morpholinopropane sulfonic acid (MOPS), pH7.4 at 4℃] and centrifuged at 1000×g for 5 minutes after each wash. The RBC pellet was resuspended in the choline chloride washing to give 40-50% hematocrit, which was also measured. A 50ul aliquot of the RBC suspension was added to 5ml 0.025% acationox (metal free detergent, Scientific products, McGraw Park, Illinois, USA) to be used for determination of intracellular Na concentrations.

Na-leak:

One and half ml each of erythrocyte was added to 30ml medium (150mM choline chloride, 10mM glucose, 1mM ouabain, 1mM furosemide, 10mM Tris-MOPS pH7.4 at 37℃) with or without 1mM AAPH, then mixed gently and aliquot to 10 tubes. The tubes were transferred in duplicates to an ice bath after incubation at 37℃ in a shaking water bath for 0, 10, 20, 30, 40 minutes. Tubes were subsequently centrifuged at 3000×g for 10 minutes, the supernatant was removed, and Na concentration was then measured.

Calculations:

Na-leak:

$$\frac{[\text{Na } \mu g/m\ell]}{[\text{min}]} \times \frac{[60\text{min}]}{[\text{hr}]} \times \frac{[\mu\text{mole}]}{[23\mu g]} \times \frac{[44-(4\times\text{HCT})]}{[4\times\text{HCT}]} = \text{Na mmole/}\ell\ \text{rbc/hr}$$

Intracelluar Na:

$$\frac{[\text{Na } \mu g]}{[m\ell]} \times \frac{[\mu\text{mole}]}{[23\mu g]} \times \frac{[101]}{[\text{HCT}]} = \text{Na mmole/}\ell\ \text{rbc}$$

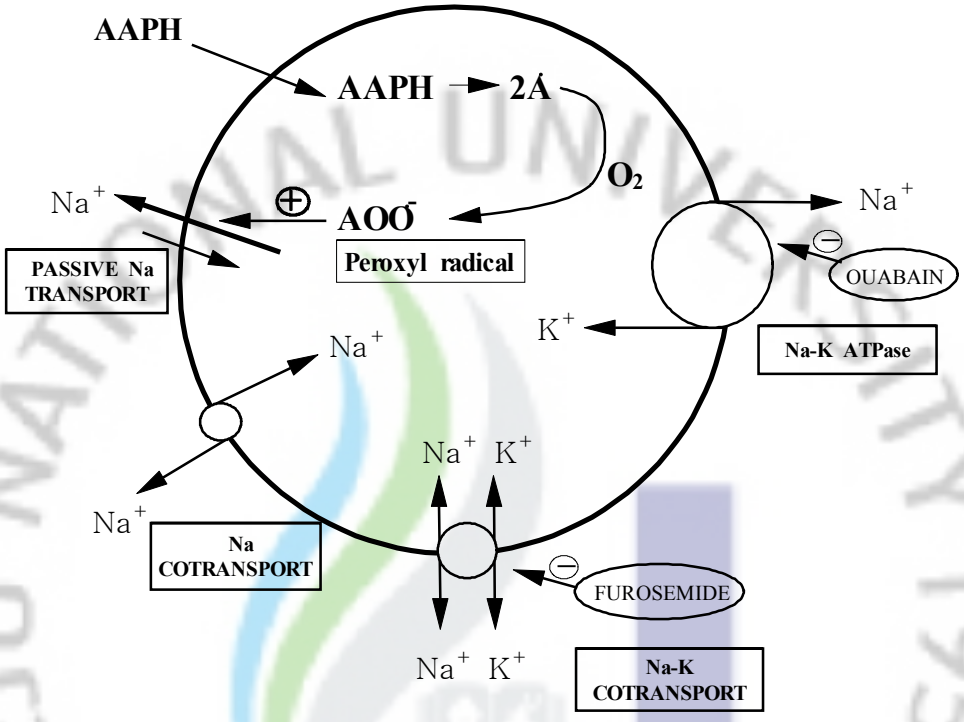

Figure 2. Model of The Mechanism of AAPH Induced Na-Leak in Red Blood Cell

5) PRP TBARS production

Platelet rich plasma (PRP) were obtained after centrifuging whole blood at 3000×g for 10 minutes. PRP TBARS (Thiobarbituric acid-reactive substances) production was measured with a modified Aust method(1978). TBARS, malondialdehyde (MDA) is an end product of fatty acid peroxidation.

An half ml PRP in 1.5ml PBS (Phosphate buffered saline) was incubated at 100℃ oil bath after adding 2ml TBA solution (15g TBA, 0.319g TCA, 1.81ml 12N HCL in 85ml D-H_2O). After cooled under tap water, the incubated mixture was centrifuged at 1000×g for 10 minutes. Finally, the TBARS in supernatant was measured with the absorbance at 532nm on spectrophotometer using deionized H_2O as blank.

6) Liver TBARS production

Aust method was used for the liver TBARS. One gram of minced liver sample in 2ml PBS was incubated at 100℃ oil bath for 20 minutes after adding 2ml TBA or nonTBA solution (0.319g TCA, 1.81ml 12N HCL in 85ml D-H_2O). The incubated mixture was cooled down and centrifuged at 1000×g for 10 minutes. The TBARS in supernatant was spectrophotometrically at 532nm using deionized H_2O as blank. All samples were performed in duplicate and the values of TBARS was the outcome subtracting the concentration of the nonTBA treated from the TBA treated.

7) Protein analysis

Liver protein was measured with Lowry method (Lowry *et al.,* 1951) using bovine serum albumin as standard protein. The absorbance for protein concentration was read at 750nm with spectrophotometer using D-H_2O as blank.

8) Measurement of hemolysis

Autohemolysis was performed by modified method of Draper *et al.* (1969). Three hundreds ul of whole blood was dispersed in 3ml PBS and centrifuged at 1000×g for 10 minutes. After removed the upper solution, the red blood cell was diluted with 3ml PBS (1:10), of which 500ul was rediluted with 4.5ml PBS with or without 1mM AAPH (1:10).

The red cell solution was incubated at 37℃ CO_2 incubator for 4 hours. After centrifugation, the supernate was spectrophotometrically read at 415nm using D-H_2O as blank. The percent of hemolysis was calculated from the absorbance of the same concentration of red blood cell hemolyzed in deionized H_2O.

9) Statistical analysis

Values were analyzed using the SAS package (SAS, 1994). Analysis of variance were conducted in a completely randomized block design. Duncan's multiple test was applied to compared individual means when F-value was significant ($p<0.05$).

Ⅲ. RESULTS & DISCUSSIONS

1. Weight gain and food efficiency

Weight gain and food efficiency are shown in Table 2. The final body weight was decreased in the *Ecklonia cava* (EC) of powder, extract and Dieckol compared with the control and significantly different between the Dieckol and the control ($p<0.05$). The feed efficiency ratio (FER) and average daily weight (ADG) were tended to decrease in all the EC groups and there are significant differences between the control and the Dieckol in FER and ADG ($p<0.05$) without difference in average feed intake among groups. LW/BW tended to decrease in all dietary groups of EC but no statistically different.

The EC powder and Dieckol were decreased in ADG with almost the same daily feed intake, consequently showing lower FER. Unlike the EC powder and Dieckol, the EC extract had a high FER, which may be explained with its higher energy source. The EC extract is a hydrolyzed product of carbohydrase, while the Dieckol with little carbohydrate and the EC powder with intact carbohydrate do not have much of available energy source. We assumed *Ecklonia cava*, especially Dieckol may have anti-obesity effect.

Kao *et al.* (2000) reported that tea polyphenol EGCG suppressed food intake with low weight gain and improved plasma lipid profile. Kang *et al.*(2007) also showed green tea extract suppressed weight gain without reducing food intake in SD rats. In the present study, polyphenol rich Dieckol reduced weight gain with the same food intake, consequently decreasing food efficient ratio (FER). Diepvens *et al.* (2005) observed that green tea increased the resting energy expenditure and fat oxidation during weight loss in overweight females. Lambs fed the seaweed diet

consumed more feed and gained less body weight than lambs fed grass hay (Al-Shorepy *et al.*, 2001).

Seaweed such as *Ecklonia cava* and algae polyphenol such as Dieckol may have antiobese effects by inducing high metabolic rate rather than affecting food intake.

Table 2. Effects of *Ecklonia cava* powder, extract and dieckol on growth rate and feed intake in rats

	Control	*Ecklonia cava* powder	*Ecklonia cava* extract	Dieckol
Initial B.W[1)](g)	216.8 ± 32.4	229.8 ± 22.7	229.3 ± 20.2	211.9 ± 23.6
Final B.W(g)*	384.4 ± 32.9[a]	351.4 ± 35.3[ab]	373.2 ± 49.6[ab]	302 ± 36.4[b]
ADG[2)](g/d)*	5.94 ± 0.86[a]	4.34 ± 0.12[ab]	5.15 ± 0.92[a]	3.22 ± 0.41[b]
ADFI[3)](g/d)	23.55 ± 3.5	22.38 ± 3.3	22.35 ± 1.9	23.23 ± 5.8
F.E.R[4)]*	0.25 ± 0.03[a]	0.19 ± 0.04[ab]	0.23 ± 0.02[a]	0.13 ± 0.04[b]
L.W/B.W[1)](%)	5.3 ± 0.8	4.2 ± 0.7	4.8 ± 0.5	4.4 ± 0.4

[1)]BW : Body weight, LW: Liver weight
[2)]ADG : Average daily gain
[3)]ADFI : Average daily feed intake
[4)]F.E.R : Feed Efficiency Ratio
Values are means±SD of 10 rats.
*Values in the same row not sharing the same superscript differ ($p<0.05$)

2. Plasma total-cholesterol, HDL-choelsterol, triglyceride and glucose

Plasma total cholesterol, HDL-cholesterol, triglyceride and glucose are shown in Table 3. Plasma total cholesterol was decreased in the EC groups compared with the control. All EC groups were tended to decrease in total cholesterol between the control and the EC powder showing significant difference ($p<0.05$). The HDL-cholesterol was significantly decreased in rats fed the EC powder and the Dieckol compared with the control ($p<0.05$). Plasma triglyceride was not different among groups. Glucose was decreased in the EC extract, but not statistically different between each two groups.

Undigestible soluble fiber is known to decrease the risk of coronary heart disease, mainly due to its charateristics of dispersibility in water, viscosity, binding and absorptive capacity, fecal bulking and fermentability in gastrointestinal tract. The capacity of seaweed polysaccharides to lower serum cholesterol levels seems to be due to its ability to disperse in water, hold and excrete cholesterol into feces. Marine algae is composed of 25-75% dietary fiber in dry weight which are primarily soluble fiber, and physiologically active marine algae polysaccharide inhibit lipid absorption in the gastrointestinal tract (Jimenez-Escrig *et al.,* 2000).

Wong *et al.* (1999) showed that diet supplemented seaweed powders of Ulva sp. and Hypnea charoides reduced the serum total cholesterol in hypercholesterolemic rats. Ming *et al.* (2006) also reported mice fed the polysaccharide diet from Lycium barbarum tended to lower serum triglyceride. Intact EC powder in present study contains a considerable amount of mucus polysaccharide fucoidan which might cause plasma cholesterol decreased. Unlike our study, some seaweed including *Ecklonia cava* did not reduce plasma cholesterol but rather elevated it to considerably large extent (Ren *et al.,* 1994). A mixed sea weed diet did

not change HDL cholesterol in rats a cholesterol rich diet (Hideomi *et al.,* 2005) which is similar to our results where *Ecklonia cava* did not affect HDL cholesterol levels. Soluble fibers such as guar gum and pectin decreased plasma triglyceride (Grizard *et al.,* 2001), while *Ecklonia cava* in present study did not decreased plasma triglyceride. Grizard *et al.* (2001) in the same study, guar gum and pectin decreased postprandial insulin concentration without changing postprandial and fasting blood glucose. EC powder diet in present study did not affect fasting glucose level.

Table 3. Effects of *Ecklonia cava* powder, extract and dieckol on the plasma lipid levels and glucose in rats

	Control	*Ecklonia cava* powder	*Ecklonia cava* extract	Dieckol
Plasma (mg/dl)				
Total-cholesterol*	106.8 ± 21.6[a]	77.7 ± 14.1[b]	86.6 ± 12.02[ab]	88.4 ± 21.4[ab]
HDL-cholesterol*	28.5 ± 5.4[a]	24.2 ± 6.3[b]	27.6 ± 3.8[a]	21.8 ± 3.3[b]
Triglyceride	89.1 ± 11.4	85.0 ± 9.5	89.1 ± 12.2	88.1 ± 7.8
Glucose	122.4 ± 16.3	135.6 ±15.9	119.5 ± 17.4	124.0 ± 8.7

Values are means ± SD of 10 rats.

*Values in the same row not sharing the same superscript differ ($p < 0.05$)

3. Liver cholesterol and triglyceride

Liver total cholesterol and triglyceride were shown in Table 4. Liver total cholesterol was decreased in the EC powder compared with other groups ($p<0.05$), Liver triglyceride was decreased in all EC groups compared with the control and showing a significant difference between the control and the Dieckol ($p<0.05$)

Kang *et al.* (2001) reported that high viscous fiber materials such as prickly pear cactus, Undaria (Wakame) and tangerine pulp did not affect liver cholesterol, but decreased liver triglyceride in rats fed cholesterol diet. Some seaweed as well as dietary fiber, particularly polyionic fiber may interact with dietary cholesterol, leading to its excretion and subsequently lowering plasma and liver cholesterol (Abe S *et al.,* 1971; Ren *et al.,* 1994). Wong *et al.* (1999) reported plasma cholesterol lowering effect was different with different seaweeds, but liver total cholesterol was not changed with any of seaweed diet in rats fed high cholesterol diet, suggesting seaweed diets did not affect liver cholesterol like our results. Unlike liver cholesterol, *Ecklonia cava* was effective in lowering liver triglyceride in present study. Kang *et al.* (2007) reported that green tea powder and extract decreased liver triglyceride, alleviating fatty liver. It was suggested that EGCG and tea polyphenol suppressed fatty acid synthetase (FAS) gene expression consequently reducing fatty acid synthesis and induced fatty acid oxidation resulting in decreased plasma and liver triglyceride. In present study, polyphenol rich Dieckol was effective in lowering liver triglyceride without affecting plasma triglyceride, suggesting Dieckol may help release liver triglyceride into plasma.

Table 4. Effects of *Ecklonia cava* powder, extract and dieckol on the liver cholesterol and triglyceride content in rats

	Control	*Ecklonia cava* powder	*Ecklonia cava* extract	Dieckol
Liver (mg/g)				
Total-cholesterol*	25.9 ± 7.9^{ab}	24.3 ± 5.2^{b}	32.1 ± 2.7^{a}	30.5 ± 8.0^{ab}
Triglyceride*	22.8 ± 7.2^{a}	17.1 ± 5.2^{ab}	16.6 ± 5.9^{ab}	13.6 ± 5.2^{b}

Values are means ± SD of 10 rats.

*Values in the same row not sharing the same superscript differ ($p < 0.05$)

4. Whole blood platelet aggregation

Platelet aggregation and hematocrit were shown in Table 5. Hematocrit was not statistically different among groups, but the control group was tended to be higher compared with other groups. Maximum and Initial slope were decreased in all EC groups compared with the control and showed a significant difference between the control and the Dieckol ($p<0.05$).

In physiological condition, collagen and thrombin are the first inducer of platelet aggregation following trauma, and ADP, epinephrine, serotonin and thromboxane (TXA2) released during the 1st phase of aggregation cause the 2nd phase aggregation. Some of these platelet aggregation agonist are known to be associated with plasma cholesterol. It has been reported that an increased platelet function is related to hyperlipoproteinmia (Carvalho *et al.*, 1974; Jamieson *et al.*, 1985). Platelet from type II hyperlipoproteinemia were activated in response to a low concentration of ADP, epinephrine and collagen (Carvalho *et al.*, 1974). Platelet membranes from hypercholesterolemic rats had higher thrombin receptors (Jamieson *et al.*, 1985). Cholesterol-rich human platelet had hypersensitivity to TXA_2 which activates platelet aggregation (Stuart *et al.*, 1980). In present study, platelet aggregation was not quite correlated with plasma cholesterol level. Naseem *et al.* (1999) observed that platelet aggregation and its release reaction were affected by the condition of oxidative stress. Kantonis *et al.* (2006) also reported that platelet activating factor (PAF) antagonists in polyphenol from olive oil exerted significant antitherosclerotic activity in rabbits. In *invitro* experiment, PAF actyltransferase in PAF biosynthesis was increased by LDL-choelsterol and decreased by tea catechin (Sugatani *et al.*, 2004). Four weeks supplementation of acute dose of 234mg/d flavanols and oligoprocyanidin from cocoa inhibited ADP and collagen-induced whole blood platelet

aggregation in human (Murphy *et al.*, 2003). A plant extract of *Yucca schidigera* inhibited thrombin induced platelet aggregation in pig platelet rich plasma (PRP) and decreased platelet adhesion to collagen and fibrinogen (Olas *et al.*, 2003). Hideomi *et al.* (2005) reported that diet with mixture of seaweed significantly decreased the ADP-induced and collagen-induced aggregation by 10-15% compared to the control. In present study, platelets of rat fed polyphenol rich Dieckol showed a decreased platelet aggregation without any change with intact EC powder or hydrolyzed extract, suggesting phenolic compounds of *Ecklonia cava* might have antiplatelet action.

Table 5. Effects of *Ecklonia cava* powder, extract and dieckol on hematocrit and platelet aggregation in rats

	Control	*Ecklonia cava* powder	*Ecklonia cava* extract	Dieckol
Hematocrit(%)	43.9 ± 3.1	41.5 ± 1.9	41.1 ± 2.1	42.3 ± 1.7
Platelet Aggregation				
Maximum(Ω)[1)*]	11.6 ± 2.2[a]	10.5 ± 1.8[ab]	10.0 ± 1.7[ab]	8.1 ± 1.8[b]
Initial Slope(Ω/min)[2)*]	12.7 ± 3.1[a]	11.0 ± 3.2[ab]	10.8 ± 1.2[ab]	6.9 ± 2.1[b]

1) Maximum aggregation is ohm at the point where aggregate dissociated.

2) Initial slope ohm change for the first one minute of aggregation.

Values are means ± SD of 10 rats.

*Values in the same row not sharing the same superscript differ ($p<0.05$)

5. Hemolysis and erythrocyte Na leak

Hemolysis and erythrocyte were shown in Table 6. Hemolysis with or without AAPH was decreased in EC extract group compared to other groups ($p<0.05$). Intracellular Na of group with *Ecklonia cava* was lower than the control, but was not statistically difference. Na leak without AAPH treated was not different among groups. Na leak in AAPH treated was increased in EC extract group compared with other groups ($p<0.05$).

AAPH, which is used for radical production, can readily cross the cell membrane and generate free radicalsin the presence of NADH, causing damage inside the cells. (Maridonneau *et al.*, 1983).

AAPH (2 2'-azobis[2-amidino-propane] dihydrochloride), an water soluble radical initiator readily penetrates into membrane, forms AAPH derived peroxyl radical in the present of oxygen and acts on membrane lipid inside of cell, causing membrane damage and lipid peroxidation. Unlike vitamin E which is localized in the cell membrane, polyphenol such as flavonoids present in their aqueous phase can scavenge intracellular and extracellular free radicals. Some flavonoids such as flavanol appear to pass through biogical membranes. (Scarbert *et al.*, 2000). Upon exposure to radical producing system such as AAPH, erythrocyte membrane is damaged and increased in Na passive leak (Maridonneau *et al.*, 1983). In *In vitro* study using rat erythrocyte, catechin and quercetin which had comparatively high radical scavenging in DPPH system showed higher Na leak increase in PMS treated erythrocyte than hesperidin and naringin, suggesting DPPH radical scavenging activity not necessarily correlated with membrane protection from oxidative damage (Lee *et al.*, 2002). In the present study, intact erythrocyte from rats of EC extract showed the lowest in both Na passive leak and hemolysis, inferring erythrocyte membrane being more stabilized. However, after erythrocyte exposed to AAPH, hemolysis increase was the lowest in erythrocyte from rats with

EC extract, while Na leak increase was the highest in erythrocyte of EC extract, suggesting antioxidant action of EC extract may exert on RBC membrane Na passive efflux and hemolysis in different ways. Curcumin, diferuloylmethane, a yellowish food pigment showed antioxidant or pro-oxidant effects depending on the curcumin concentration in incubation medium by measuring AAPH induced intracellular K+ loss during erythrocyte lysis. Sulfated polysaccharide, fucoidan from a brown seaweed, *Laminaria japonica* inhibited complement mediated hemolysis (Zvyagintseva *et al.*, 2000) and fucoidan fraction prepared from *Ascophyllum nodosum* inhibited hemolysis through pathways of complement activation (Adachi *et al.*, 1990). Liu *et al.* (2002) reported that ginsenosides extracted from *Panax ginsen* inhibit AAPH induced erythrocyte hemolysis, of which potencies depend on the type of sugar moieties and their connective positions to the ring of triterpene dammarane in the structure of ginsenosides. From previous studies, we conclude that polyphenol rich Dieckol and bioactive polysaccharides, fucoidan in *Ecklonia cava* both may play roles in preventing erythrocyte hemolysis independently and sinergistically.

Table 6. Effects of *Ecklonia cava* powder, extract and dieckol on hemolysis and erythrocyte leak

	Control	*Ecklonia cava* powder	*Ecklonia cava* extract	Dieckol
Hemolysis(%)				
−AAPH*	3.11 ± 0.6^{ab}	3.59 ± 0.8^{a}	2.93 ± 0.8^{b}	3.43 ± 0.8^{a}
+AAPH*	51.39 ± 9.2^{a}	50.22 ± 1.4^{a}	35.11 ± 10.9^{b}	50.13 ± 2.0^{a}
Intracellular Na (mmol/1RBC/hour)	2.86 ±0.73	2.00 ± 0.85	2.51 ± 0.66	2.49 ± 1.04
Erythrocyte Na leak (mmol/1RBC/hour)				
−AAPH	0.42 ± 0.08	0.49 ± 0.16	0.39 ± 0.12	0.49 ± 0.11
+AAPH*	0.76 ± 0.13^{b}	0.96 ± 0.15^{b}	1.32 ± 0.26^{a}	0.89 ± 0.12^{b}
△ Na leak	0.34 ±0.07	0.47 ±0.1	0.93 ±0.12	0.40 ±0.08

Values are means ± SD of 10 rats.
*Values in the same row not sharing the same superscript differ($p < 0.05$)

6. Liver and PRP TBARS production

Liver and PRP TBARS production were shown in Table 7. PRP TBARS was decreased in all EC groups compared with the control and showed a significant difference between the control and the Dieckol. Liver TBARS was not statistically different among groups, but the control group was tended to be higher compared with other groups.

Wine polyphenol suppressed generation of copper and AAPH induced LDL oxidation in Chinese hamster ovary cell (Fremont *et al.*, 1999). Curosawa *et al.* (2005) observed that rabbit fed diet containing cacao liquor polyphenol had a decreased plasma TBARS and a reduced athrosclerotic lesion in aorta compared to that of the control group. Polyphenol rich extracts from plants such as rosemary, grape, citrus and marigold reduced plasma MDA production, and rosemary and grape especially was effective in suppressing conjugated diene formation in plasma of rats fed a $\omega-3$ fat (Gladine *et al.*, 2007). Goat fed alfalfa diet supplemented seaweed showed much lower lipid peroxidation compared to the controls, concomitantly lower plasma cortisol during transport stress (Kannan *et al.*, 2007). Saker *et al.* (2004) also reported that forage feed containing brown seaweed enhanced antioxidant status and immune function in heat stressed male lambs. Subcutaneous injection of fucoidan from a brown algae, *Fucus vesiculosus* had protective role in oxalate induced free radical injury on renal cell, restoring renal Na-K ATPase and decreased plasma TBARS levels in Wistar rats (Coothan *et al.*, 2006). In present, diets with EC powder, extract and Dieckol decreased liver TBARS production in some extent, but TBARS in PRP was more effectively suppressed by diets with *Ecklonia cava* especially polyphenol rich Dieckol. With results from previous research, we suggest that bioactive compounds of *Ecklonia cava.* phlorotannin and fucoidan both have health promoting effects with their antioxidant properties, reducing oxidative stress and aging.

Table 7. Effects of *Ecklonia cava* powder, extract and dieckol on the PRP and liver TBARS productions in rats

	Control	*Ecklonia cava* powder	*Ecklonia cava* extract	Dieckol
TBARS				
PPR[1)*] (mmol/ml plasma)	5.27 ± 1.6[a]	4.43 ± 1.7[ab]	3.69 ± 1.5[ab]	3.09 ± 1.8[b]
Liver (mmol/g protein)	6.44 ± 1.7	5.25 ± 1.4	5.71 ± 1.2	5.45 ± 1.0

Values are means ± SD of 10 rats.
*Values in the same row not sharing the same superscript differ ($p<0.05$)
1) PRP : platelet rich plasma

7. GOT & GPT

GPT and GOT were shown in Table 8. GPT was decreased in *Ecklonia cava* groups compared with the control and the lowest group is EC powder with statistically difference among groups ($p<0.05$). GOT was shown the same result that EC powder was the lowest among groups and with statistically difference ($p<0.05$).

GPT(ALT) is produced and found mainly in liver, and small amounts found in the heart, muscle and kidney, therefore GPT is more for the index of liver disease, while GOT(AST) is found in many body tissues including the heart, muscle, kidney, brain, lung and also liver. When the liver is injured or inflamed, the level of GPT in blood usually rise, and body tissue such as heart and liver is damaged GOT usually rise. Elhalwagy *et al.* (2008) reported that Green tea polyphenol had prophylactic effect on an insecticide, fenitrothion induced liver and kidney injury, reducing the elevated plasma GOT and GPT, urea, creatine and MDA production in albino rats. Fucoidan from Sigma chemicals alleviated CCl^4 induced liver fibrosis, decreasing plasma GPT and GOT (Hayashi *et al.*, 2008). Similarly, Wong *et al.* (2000) reported that aqueous extracts of brown algae, *Myagropsis myagroides* and *Sargassum henslowianum* prevented CCl^4 induced liver damage, decreasing plasma GOT and GPT. In present study, supplementation of cholesterol may cause fatty liver in all groups of rat, presumably resulting in increased plasma GPT, but GPT of all group of rats fall in normal range. Intact EC powder which contains polyphenol and fucoidan is the most effective in lowering GPT. Unlike GPT, GOT levels in groups of the control and EC powder fall on upper limit and was further increased in EC extract and Dieckol. Thus, we can assume that EC powder is effective of inhibiting inflammation of liver.

Table 8. Effects of *Ecklonia cava* powder, extract and dieckol on the plasma GOT & GPT in rats

	Control	*Ecklonia cava* powder	*Ecklonia cava* extract	Dieckol
		Unit/l plasma		
GPT*	18.6 ± 9.4^{a}	8.5 ± 2.3^{b}	16.9 ± 6.7^{ab}	13.4 ± 4.5^{ab}
GOT*	59.6 ± 12.4^{b}	54.5 ± 9^{b}	74.3 ± 15^{ab}	80.1 ± 21.6^{a}

1)GPT : serum glutamate-pyruvate transferase

2)GOT : serum glutamate-oxalate transferase

Values are means ± SD of 10 rats.

*Values in the same row not sharing the same superscript differ ($p<0.05$)

Ⅳ. CONCLUSIONS

This study was carried out to compare the effect of 10% EC powder, 5% EC extract and 3% Dieckol on antiobese, hypocholesterolemic, antiplatelet and antioxidant in rats fed 5% cholesterol diets for four weeks.

1. The final body weight was decreased significantly in rats fed Dieckol compared with the control and with low food efficiency ($p<0.05$).

2. Plasma total cholesterol and was significantly decreased in group of EC powder compared with other groups ($p<0.05$) and HDL-cholesterol was significantly decreased in Dieckol and EC powder compared to the control ($p<0.05$).

3. Liver triglyceride was significantly decreased in group of Dieckol compared with other groups ($p<0.05$)

4. Platelet aggregation in the maximum and initial slope were significantly decreased in Dieckol compared with the control (both, $p<0.05$).

5. Hemolysis both with or without AAPH treatment was significantly decreased in EC extract compared with other groups ($p<0.05$) but erythrocyte Na leak increase was significantly increased in EC extract compared with other groups ($p<0.05$).

6. Platelet rich plasma (PRP) and liver TBARS production were decreased in all EC groups, and PRP TBARS was significantly decreased in group of Dieckol compared with the control ($p<0.05$).

7. GOT and GPT compared with the control was significantly decreased in rats fed EC powder ($p<0.05$).

In conclusion, consumption polyphenol rich Dieckol of *Ecklonia cava* has some favorable effects in weight gain, platelet aggregation, RBC membrane stability and lipid peroxidation. In addition, EC powder is effective in lowering plasma total cholesterol, GPT and GOP. Intact EC powder rich in polysaccharide and polyphenol might have health benefits for human as a potential functional food.

V. REFERENCES

Arai Y, Watanabe S, Kimira M, Shimoi K, Kinae N (2000). Dietary intakes of flavonols, Flavones and isoflavones by Japanese women and the inverse correlation between quercetin intake and plasma LDL cholesterol concentration. *J Nutr* 130:2243-2250.

Abe S & Kaneda T(1971) The effect of edible seaweeds on choelsterol metabolism in rats. *Proc Int Seaweed Symp.* 1971:39:239.

Adachi, Y., Ohno, N., Ohsawa, M., Oikawa, S., Yadomae, T., (1990). Macrophage activiation *in vitro* by chemically cross-linked (1,3)-β-D glucans. *Chem. Pharm, Bull.* 38, 988-992.

A. Jimenez-Escrig, F. J. Sanchez-Muniz (2000). Dietary fibre from edible seaweeds: chemical structure. Physicochemical properties and effects on cholesterol metabolism. *Nutrition Research* 20(4):585-598.

Arnab Banerjee, A. Kunwar, B. Mishra, K.I. Priyadarsini (2008). Concentration dependent antioxidant/pro-oxidant activity of curcumin studies from AAPH induced hemolysis of RBCs. *Chemico-Biological Interactions.* 174:134-139.

Broncel M, Balcerak M, Sikora J & Chojnowska-Jezierska J (2007). Effect of fluvastatin extended release on the protein-lipid structure on erythrocyte membrane and C-reactive protein in patients with hyperlipidemia. *Polski merkuriusz lekarski* 22:107-111.

Buege, J. A. and Aust, J, D. (1978) Microsomal lipid peroxidation. *Methods Enzymol.* 52,302-310.

Bo Li*, Fei Lu, Xinjun Wei and Ruixiang Zhao (2008). Fucoidan:

Structure and Bioactivity. *Molecules* 13, 1671-1695.

C.K. Wong, V.E.C. Ooi*, P.O. Ang (2000). Protective effects of seaweeds against liver injury caused by carbon tetrachloride in rats. *Chemosphere* 41:173-176.

C. Glandine, C. Morand, E. Rock, D. Bauchart, D. Durand (2007). Plant extracts rich in polyphenols (PERP) are efficient antioxidants to prevent lipoperoxidation in plasma lipids from animals fed *n*-3 PUFA supplemented diets. *Animal Feed Science and Technology.* 136:281-296.

Coothan K. Veena, Anthony Josephine, Sreenivasan P. Preetha, Palaninathan Varalakshmi, Rajaguru Sundarapandiyan (2006). Renal peroxidative changes mediated by oxalate: The protective role of function. *Life Science* 79:1789-1795.

Carvalho A.C.A, Colman R.W, Less R.S. (1974). Platelet function in hyperlupoproteinemia. *N, Engl. J. Med.* 290:434-438.

Chew DP, Bhatt DL & Topol EJ (2001). Oral glycoprotein IIb/IIIa inhibitors: why don't they work? *Am J Cardiovasc Drugs* 1(6):421-428.

Chaudhuri S, Banerjee A, Base K, Sengupta B, Pradeep K (2007). Interaction of flavonoids with red blood cell membrane lipids and proteins: antioxidant and antihemolytic effects. *International J Biol. Macromol.* 41(1):42-48.

Draper HH & Callant AS (1969). A simplified hemolysis test for vitamin E deficiency. *J Nutr* 98:390-394.

Diepvens K, Kovacs EMR, Nijs IMT, Vogels N, Westerterp-Platenga MS (2005). Effect of green tea on resting energy expenditure and substrate oxidation during weight loss in overweight females. *Br J*

Nutr 94:1026-1034.

Fremont L, Gozzelino MT, Franchi MP, Linard A (1998).Dietary flavonoids reduce lipid peroxidation in rats fed polyunsaturated or monounsaturated fat diets. *J. Nutr.* 128(9):1495-1502.

Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissue. *J Biol Chem,* 1957;226(1):497-509.

G. Kannan, K.E. Saker, T.H. Terrill, B. Kouakou, S. Galipalli, S. Gelaye (2007). Effect of seaweed extract supplementation in goats exposed to simulated preslaughter stress. *Small Ruminant Research* 73: 221-227.

Grizard D, Dalle M & Barthomeu C (2001) Changes in insulin and corticosterone levels may partly mediate the hypolipidemic effect of guar gum and low molecular weight pectin in rats. *Nutrition Res.* 21:1185-1190.

Heinrich M & Prieto JM (2008). Diet and healthy ageing 2100: Will we globalise local knowlegae systems? Ageing Res Reviews 7(3):249-274.

Huang ZX, Mei XT, Lv JY (2005). Protective effects of polysaccharide of Spirulina platensis and Sargassum thunbeergii on vascular of alloxan induced diabetic rats. *Zhongguo Zhong Yao Za Zhi* 30(3):211-215.

Hubbard GP, Wolffram S, de Vos R, Bovy A, Gibbins JM & Lovegrove JA (2006). Ingestion of onion soup high in quercetin inhibits platelet aggregation and essential components of the collagen-stimulated platelet activation pathway in man: a pilot study. *Br J Nutr.*

96(3):482-488.

Haralabos C, Karantonis, Smaragdi Antonopoulou, Despina N, Perrea, Dimitrios P. Sokolis, Stamations E. Theochairs, Nikolaos Kavantzas, Dimitrios G. lliopoulos, Constantions A. Demopoulos (2006). *In vivo* antiatherogenic properties of olive oil and its constituent lipid classes in hyperlipidemic rabbits. *Nutrution, Metabolism & Cardiovascular Diseases* 16,174-185.

Hideomi Amano, Makoto Kakinuma, Daniel A Coury, Haruka Ohno, Takaaki Hara (2005). Effect of a seaweed mixture on serum lipid level and platelet aggregation in rats. *Fisheries Science* 71:1160-1166.

Hara H, Haga S, Aoyama Y & Kiriyama S (1999). Short-Chain Fatty Acids Suppress Cholesterol Synthesis in Rat Liver and Intestine. *J Nutr* 129:942-948.

Hara H, Haga S, Kasai T & Kiriyama S (1998). Fermentation Products of Sugar-Beet Fiber by Cecal Bacteria Lower Plasma Cholesterol Concentration in Rats. *J Nutr* 128(4):688-693.

Helena BL. Sang KN, Sung IK (2002). Green tea extract inhibits the lymphatic absorption of cholesterol and α-tocopherol in ovariectomize rats. *Journal of Nutrition.* 132(6):1282-1288.

Jerzy Juskiewicz, Zenon Zdunczyk, A. Jurgoniski, Ewa Zary-Sikorska (2007). Extract of green tea leaves partially attenuates streptozotocin-induced changes in antioxidant status and gastrointestinal functioning in rats. *Nutrition Research* 28: 343-349.

Jamieson G.A, Tandon N.N, Hoeg J.M. (1985). Perfusion studies on the formation of mural thrombi with cholesterol-modified and hypercholesterolemic platelets. *J. Lab. Clin. Med.* 105:157-163.

Ji Hyun Lee, Young Hee Kang and JS Kang (2002). Free radical scavenging of flavonoids and their effects on erythrocyte Na leak, platelet aggregation and TBARS production. *Korean J Nutrition.* 5(4):197-202.

Keevil JG, Osman HE, Reed JD and Folts JD (2000). Grape fruit, but not orange juice or grapefruit juice inhibit human platelet aggregation. *J Nutr* 130:53-56.

K.H. Wong, S. W. Sam, P.C.K. Cheung (1999). Changes in lipid profiles of rats fed with seaweed-based diets. *Nutrition Res.* 19;10:1519-1527.

Kao YH, Hiipakka RA, Liao S (2000). Modulation of endocrine systems and food intake by green tea epigallocatechi gallate. *Endocrynology* 141:980-987.

Kang JA, Kang JS (2008). Effect of green tea on weight gain, plasma and liver lipids and lipid peroxidation in pair fed rats. *Korean J Nutrition.* 41(7):602-611.

Kang MS, Kang JS (2001). Effect of tangerine pulp, sea tangle or prickly pear cactus on lipid level, intestinal cholesterol absorption, platelet aggregation and liver tissue in hypercholesterolemic rats. *Korean J Nutrition.* 34(2):141-149.

K. H. Wong, S. W. Sam, P. C. K. Cheung, P.O. Ang (1999). Changes in l ipid profiles of rats fed with seaweed-based diet. *Nutrition Research* 19(10):1519-1527.

Kelley J.J, Tsai A.C (1978). Effect of pectin, gum arabic and agar on cholesterol absorption, synthesis, and turnover in rats. *J. Nutr.* 108:630-639.

Lavy A, Fuhrman B, Markel A, Dankner G, Ben-Amotz A, Presser D, Aviram M (1994). Effect of dietary supplementation of red or white wine on human blood chemistry, hematology and coagulation favorable effect of red wine on plasma high-density lipoprotein. *Ann Nutr Metab* 38(5):287-294.

Lambo-Fodje AM, Oste R & Nyman ME (2006). Short-chain fatty acid formation in the hindgut of rats fed native and fermented oat fibre concentrates. *Br J Nutr 96(1):47-55.*

Lowry, O.H., Rosebrough, N. J., Farr, A. L. and Randoll, F. J (1951). Protein measurement with folin phenol reagent. *Bio. Chem.* 193,265-273.

Lucie Fremont, Leila Belguendouz and Serge Delpal (1999). Antioxidant activity of resveratrol and alcohol-free wine polyphenols related to LDL oxidation and polyunsaturated fatty acids. *Life Science* 64,26:2511-2521.

Lin JK & Lin-Shiau SY (2006) Mechanism of hypolipidemic and antiobesity effects of tea and tea polyphenol. *Mol Nutr Food Res.* 50(2):211-217

Manal E.A. Elhalwagy, N. S. Darwish, E. M. Zaher (2008). Prophylactic effect of green tea polyphenols against liver and kidney injury induced by fenitrothion insecticide. *Pesticide Biochemistry and Physiology* 91:81-89.

Marco, D. B. F., Joseph, V., & John, K (1997). Mechanisms of disease:

antioxidants and atherosclerotic heart disease. *New England Journal of Medicine,* 337(6), 408-416.

Maridonneau I, Braquet P, Garay RP (1983). Na+ and K+ transport damage induced by oxygen free radicals in human red blood cell membranes. *Biol Chem.* 285:3107-3113.

Ma Ming, Liu Guanhua, Yu Zhanhai*, Chen Guang, Zhang Xuan (2006). Effect of the Lycium barbarum polysaccharides administration on blood lipid metabolism and oxidative stress of mice fed high-fat diet *in vivo*. *Food Chemistry* 113:872-877.

Murakai K, Mitani S, Ohara K & Nagaoka SI (2005). Structure activity relationship of the tocopherol regeneration reaction by catechins. *Free Radical Biol Med.* 38(1)1243-1256.

Murphy KJ, Chronopoulos AK, Singh I, Francis MA, Pike MJ & Sinclair AJ (2003). Dietary flavanols and procyanidin oligomers from cocoa (*Theobroma cacao*) inhibit platelet function. *Am J Clin Nutr* 77(6):1466-1473.

Neseem KM & Bruckdorfer KR (1999). The influence of organic peroxides on platelet aggregation and sensitivity to nitric oxide. *Platelets* 10:146-152.

Olas B, Wachowicz B, Stochmal A & Oleszek W (2003). Inhibition platelet adhesion and secretion by different phenolics from *Yucca schidigera* Roezl. *Bark. Nutrition* 2005:199-206.

Pckham M.A, Mustard J.K (1986). The role of platelets in the development and complication of atherosclerosis. *Seminars in Hematology.* 23;1:8-26.

Pignatelli P, Pulcinelli P, Violi F (2000). The flavonoids quercetin and catechin synergicall inhibit platelet function by antagonizing the intracellular production of hydrogen peroxide. *Am J Clin Nutr*. 72:1150-1155.

Ryszawa N, Kawczynska-Drozdz A, Adamek-Guzik T, Korbut R & Guzik TJ (2006). Effects of novel plant antioxidants on platelet superoxide production and aggregation in atherosclerosis. *J Physiol Pharmacol 57(4):611-626.*

Ren D, Noda H, Amano H, Nishino T, Nisizawa K (1994). Study on antihypertensive and antihyperlipidemic effects of marine algae. *Fish Sci* 1994; 60:83-88.

Suheeta Roy, Hedley C. Freake, Maria Luz Fernandez (2002). Gender and hormonal status affect the regulation of hepatic cholesterol 7α-hydroxylase activity and mRNA abundance by dietary soluble fiber in the guinea pig. *Atherosclerosis* 163:29-37.

Sugatani J, Fukazawa N, Ujihara K, Yoshinari K, Noguchi H & Miwa M (2004). Tea polyphenols inhibit acetyl-CoA:1-alkyl-sn-glycero-3-phosphocholine acetyltransferase (a key enzyme in platelet-activating factor biosynthesis) and platelet-activating factor-induced platelet aggregation. *Int Arch Allergy Immunol.* 134(1):17-28

Stary HC, Chandler AB, Glagov S, Guyton JR, insull Jr. W, Rosenfied ME, Schaffer SA, Schwartz CJ, Wagner WD, Wissier RW (1994). A definition of initial fatty streak, and intermediate lesions of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, *American Heart Association.*

Circulation 89:2462-2478.

Shefer S.H.S, Lapar V, Mosbach E.H (1973). Regutory effects of dietary sterols and bile acids on rat intestinal HMG-CoA reductase. *Lipid Res* 14:400-405.

S. Hayashi, A. Itoh, M. Kawase, Kiyohito Yagi (2007). Fucoidan partly prevents CCl_4- induced liver fibrosis. *European Journal* 580: 380-384.

Salah N, Miller NJ, Bolwell GP, Rice-Evan C (1995). Polyphenolic flavanols as scavengers of aqueous phase radical and as chain breaking antioxidants. *Arch Biochem Biophys.* 322:339-346.

Sobolova L, Skottoya N, Vecera R, urbanek K (2006). Effect of silymarin and its polyphenol fraction on cholesterol absorption in rats. *Pharmacol Research* 25:425-433.

Saker, K.E., Fike, J. H., Veit, H., Ward, D.L. (2004). Brown seaweed (Tasco^{TM}) treated conserved forage enhances antioxidant status and immune function in hear-stressed wether lambs. J. *Anim. Physiol. Anim. Nutr.* 88(3-4),122-130.

Stuart M.J, Tepper SA, Kritchevsky D (1974). Influence of synthetic conjugates of cholic acid on cholesteremia in rats. *J. Nutr.* 104:1185-1188.

S.A. A1-Shorepy, G.A. Alhadrami, I.A. Jamali (2001). Effect of feeding diets containing seaweed on weight gain and carcass characteristics of indigenous lambs in the United Arab Emirates. *Small Ruminant Research* 41:283-287.

Scarbert A, Williams G (2000) Dietary intake and bioavailability of polyphenols. *J. Nutr* 130:2073S-2085S,

Terao J, Pikula M, Yao Q (1994). Protective effect of epicatechin, epicatechin gallate and quecertin on lipid peroxidation in phospholipid bilayers. *Arch Biochem Biophys.* 308(1):278-284.

Tohru Kurosawa, Fumi Itoh, Aiko Nozaki, Yoshihisa Nakono, Shin-ichiro Katsuda, Naomi Osakabe, Hirokazu Tsubone, Kazuo Kondo, Hiroshige Itakura (2005). Suppressive effects of cacao liquor polyphenols (CLP) on LDL oxidation and the development of atherosclerosis in Kurosawa and Kusanagi-hypercholesterolemic rabbits. *Atherosclerosis* 179:237-246.

T. Kayashima, T. Katayama (2002). Oxalic acid is available as a natiural antioxidant in some systems. *Biochim. Biophys. Axta* 1573:1-3.

Tatiana N, Zvyagintseva, Natalia M, Shevchenko, Irina V. Nazarova, Andrey S. Scobun, Pavel A. Luk'yanov, Ludmila A. Elyakova. (2000). Inhibition of complement activation by water-soluble polysaccharides of some far-easten brown seaweeds. *Comparative Biochemistry and Physiology Part C* 126:209-215.

Weiss H.J, M. Progres (1975). Platelet physiology and abnormalities of platelet function. *N. Engl. J. Med.* 29:531-541.

Wang, J., Zhang, Q., Zhang, Z., & Li, Z (2008). Antioxidant activity of sulfacted polysaccharide fractions extracted from Laminaria japonica. *International Journal of Biological Macromolecules,* 42(2), 127-132.

Xiaoping Yuan, Jing Wang, Huiyuan Yao, Feng Chen (2004). Free radicals-scavenging capacity and inhibitory activity on rat erythrocyte hemolysis of feruloyl oligosaccharides from wheat bran insoluble dietary fiber. *LWT* 38:877-883.

Yoon YH, Chung HY & Choi JS (2008). Anti-hyperlipidemic effect of an edible brown algae, Ecklonia stolonifera and its constituents of poloxamer 407 induced hyperlipidemic and cholesterol fed rats. *Arch Pharm Res*. Dec;31(12):1564-1571.

Yu PH, Li N. & Li PC (2003). Antihyperlipidemic effects of different molecular weight sulfated polysaccharides from Ulva Pertusa (Chlorophyta). *Pharmacological research* 48:543-549.

Yang HL, Chen SC, Chang NW, Lee ML Wang HH, Hseu YC (2006). Protection from oxidative damage using bidens pilosa extracts in normal human erythrocyte. *Food and Chemical toxicology* 44: 1513-1521.

Zai-Qun Liu, Xu-Yang Luo, Yun-Xiu Sun, Yan-Ping Chen, Zhi-Cai Wang (2002). Can ginsenosides protect human erythrocytes against free-radical-induced hemolysis? *Biochimica et Biophysica Acta* 1572:58-66.