



International Journal of Pharmacology

ISSN 1811-7775

RESEARCH ARTICLE

OPEN ACCESS

DOI: 10.3923/ijp.2015.798.805

Clinical Trial of the Hypolipidemic Effects of a Brown Alga *Ecklonia cava* Extract in Patients with Hypercholesterolemia

¹Eun-Kyung Choi, ¹Soo-Hyun Park, ²Ki-Chan Ha, ¹Soon-Ok Noh, ¹Su-Jin Jung, ³Han-Jung Chae, ^{1,3}Soo-Wan Chae and ⁴Tae-Sun Park

¹Clinical Trial Center for Functional Foods, Chonbuk National University Hospital, Jeonju, Republic of Korea

²Healthcare Claims and Management Incorporation, Jeonju, Republic of Korea

³Department of Pharmacology, Chonbuk National University Medical School, Jeonju, Republic of Korea

⁴Department of Internal Medicine, Chonbuk National University Medical School, Jeonju, Republic of Korea

ARTICLE INFO

Article History:

Received: July 01, 2015

Accepted: August 22, 2015

Corresponding Authors:

Soo-Wan Chae

Clinical Trial Center for Functional Foods, Chonbuk National University Hospital, Jeonju, Republic of Korea

Tae-Sun Park

Department of Internal Medicine, Chonbuk National University Medical School, Jeonju, Republic of Korea

ABSTRACT

Ecklonia cava, an edible brown alga, is recognized as a rich source of polyphenols and the lipid-lowering effects of which have not yet been studied well. We investigated the efficacy and safety of *Ecklonia cava* extract (ECE) on blood lipid profiles in subjects with hypercholesterolemia. Eighty healthy subjects with more than 200 mg dL⁻¹ of total cholesterol or more than 110 mg dL⁻¹ of LDL-cholesterol level were randomly assigned to receive ECE (n = 40) or placebo (n = 40) for 12 weeks. Changes in serum total cholesterol, LDL-cholesterol, HDL-cholesterol, triglyceride and waist-hip ratio levels were measured after the 12-week intervention. The ECE significantly lowered total cholesterol level by 2.8% and LDL-cholesterol level by 11.1% after 12 week supplementation as compared to the placebo (p = 0.039, 0.030, respectively). The HDL-cholesterol level did not change in the ECE group, whereas an increase of 5.6% was observed in the placebo group with a significant difference between groups (p = 0.021). No corresponding changes were seen for triglyceride and waist-hip ratio levels between the two groups. There was no causal relationship between the ingestion of ECE and adverse drug reactions. We found that ECE supplementation improved blood lipid profiles through decreasing total cholesterol and LDL-cholesterol levels which are known as major cardiovascular risk factors. This result suggests that ECE supplementation may be effective in the prevention and treatment of atherosclerotic cardiovascular diseases but more studies are needed to establish the long-term safety and effectiveness.

Key words: Cardiovascular risk, cholesterol, dieckol, *Ecklonia cava*, lipid, brown algae, phlorotannin, polyphenol

INTRODUCTION

It is widely acknowledged that high blood cholesterol levels are one of the major risk factors for atherosclerosis and cardiovascular disease (CVD) and lowering in Low-Density Lipoprotein (LDL)-cholesterol level reduces the incidence of CVD (Kannel *et al.*, 1979; Gordon *et al.*, 1981; Lipid Research Clinics Programme, 1984; Heart Protection Study

Collaborative Group, 2002). Thus, lowering cholesterol level is considered as a primary target to reduce cardiovascular risks (National Cholesterol Education Program, 2001) and for this goal, lifestyle modification through changing dietary patterns and physical activities has been emphasized (Eckel *et al.*, 2014).

However, many people with cardiovascular risks are unable or unwilling to modify their lifestyle and often fail to

adhere to behavioral treatments. This represents an “Unmet medical need” for subjects with hypercholesterolemia. As a result, prescription of lipid-lowering drugs has been a main strategy used to reduce blood cholesterol levels. However, even 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors that are known to be highly effective among several lipid-lowering agents (Heart Protection Study Collaborative Group, 2002; Nissen *et al.*, 2004), may be associated with serious side effects (Joy and Hegele, 2009; Abd and Jacobson, 2011). In this regard, it is necessary to develop other safe therapies with different modes of action and it is accepted that dietary supplements may be used as alternatives in cases of intolerance or adverse effects, or in combination with statins when the objectives have not been achieved (Gylling *et al.*, 2014).

Polyphenols, naturally present in vegetables and fruits, are widely known to contribute to promotion of health. Many clinical studies report that terrestrial polyphenols, such as catechins in green tea (Nagao *et al.*, 2007; Brown *et al.*, 2009), flavonols in cacao (Grassi *et al.*, 2005; Davison *et al.*, 2008) and quercetin in fruits and vegetables (Knekt *et al.*, 1996; Edwards *et al.*, 2007) improve metabolic syndrome and reduce cardiovascular risks.

Polyphenols are also known to be abundant in marine macroalgae (seaweed), which have long been used as foodstuffs and folk remedies in Asian countries. Polyphenols are found in higher concentrations particularly in brown algae (Phaeophyta) rather than in green (Chlorophyta) and red algae (Rhodophyta) (Llorente-Mirandes *et al.*, 2011). phlorotannin, which are polyphenolic secondary metabolites, are formed by polymerization of phloroglucinol (1,3,5-trihydroxybenzene) and are isolated only from brown algae (Wang *et al.*, 2012). They are found in higher levels in *Ecklonia cava* (EC) relative to other brown algae and are thought to be key bioactive compounds among the ingredients in brown algae (Heo *et al.*, 2005). Recent studies have reported that phlorotannins have anti-oxidant (Ahn *et al.*, 2007; Shibata *et al.*, 2008), anti-cancer (Harada and Kamei, 1997), anti-diabetic (Kang *et al.*, 2010), anti-lipidemic (Yoon *et al.*, 2008; Yeo *et al.*, 2012), anti-hypertensive (Hyun *et al.*, 2006), anti-HIV-1 (Ahn *et al.*, 2004), anti-allergic (Shim *et al.*, 2009) and matrix metalloproteinase inhibition activities (Kim *et al.*, 2006).

The above effects suggest that polyphenols or phlorotannin of brown algae are possible to modulate lipid metabolism or cardiovascular risks in humans. Only a few animal studies (Yoon *et al.*, 2008; Yeo *et al.*, 2012) and one Randomized Clinical Trial (RCT) (Shin *et al.*, 2012) have been reported regarding the anti-lipidemic effects of these materials, although studies on marine algal polyphenols have increased exponentially since the 1970s, when reports on them and their tannin characteristics began to appear (Martinez and Castaneda, 2013).

In this study, we investigated the lipid-lowering effects and safety of the extract from a brown alga EC in mild hypercholesterolemic subjects by employing a randomized, double-blind and placebo-controlled design.

MATERIALS AND METHODS

Ethnic statements: The study was conducted according to the Helsinki Declaration and guideline for Good Clinical Practice. The study protocol and informed consent form were approved by the Functional Foods Institutional Review Board (IRB) of Chonbuk National University Hospital (FFIRB number, 2010-02-018; the date of approval, February 10th 2011). All volunteers gave written informed consent before the study began. An independent contract research organization (Healthcare Claims and Management Co., Ltd., South Korea) was responsible for monitoring the study according to Good Clinical Practice. The protocol is registered at www.ClinicalTrials.gov (NCT02091024).

Study design: This study was a 12 week, randomized, double-blind and placebo-controlled trial conducted at the Clinical Trial Center for Functional Foods (CTCF2) in Chonbuk National University Hospital. Treatment arms were comprised of a test group and a placebo group and a 1/1 randomization was performed. The test group consumed 400 mg of extract of EC (ECE) a day (200 mg, twice a day) for 12 weeks and the control group consumed the placebo.

The subjects visited the CTCF2 four times during the 12 week intervention period: at the screening visit, visit 1 (randomization), visit 2 (after 6 weeks) and visit 3 (after 12 weeks, end of the study). For checking eligibility of participants, evaluations including comprehensive physical examination, medical history taking, electrocardiography (ECG), blood laboratory tests and anthropometric measurements were performed at the screening visit. Primary outcomes including total and LDL-cholesterol levels and secondary outcomes including High-Density Lipoprotein (HDL)-cholesterol, triglyceride and Waist-Hip Ratio (WHR) levels were measured at the screening visit (baseline), visit 2 and visit 3. Safety parameters including adverse events, vital signs, physical examination (at the visits 1, 2 and 3), ECG and blood laboratory tests (at the screening visit and visit 3) were assessed.

During the 12 week intervention period, participants were asked to keep their usual lifestyle patterns (e.g., diets, daily physical activities and sleeping habits) and were prohibited from taking any other functional foods or dietary supplements. They were also asked to immediately report any adverse events and any changes in lifestyle patterns, recent illness and medical treatments to the investigators. All of the above information was reviewed at visit 2 and 3. Test products were provided to participants at visits 1 and 2 and any remaining pills were returned at the next visit and counted one by one to assess compliance. There was no deviation from the study protocol during the intervention period.

Study participants: The eligible participants were healthy males and females aged from 19-80 years with more than 200 mg dL⁻¹ of total cholesterol or more than 110 mg dL⁻¹ of LDL-cholesterol levels. In brief, subjects were considered ineligible if they: i) took lipid-lowering agents, ii) had any

current illness or any history of chronic medical diseases in the recent past 3 years, iii) had participated in any other clinical trials with investigative medicinal products within the past 2 months and iv) were allergic or hypersensitive to any of the ingredients in the test products. Additional exclusion criteria that could risk the subject's safety or interfere with successful participation in the study included: pregnant women or women who were breast-feeding, history of alcohol or drug abuse, or abnormal laboratory tests.

The participants were recruited from the Jeonju city area through local advertisements during the year 2011. Among the 105 volunteers screened, 80 participants who fulfilled the inclusion criteria were enrolled in this study (Fig. 1).

Test supplement: The test products were supplied by BotaMedi Inc. (Jeju, South Korea). Standardization of ECE was ensured in the production process. The ECE powder was yielded from dried EC through three phases: extraction by ethanol, concentration and a freeze-drying process. The 8.2% of dieckol as an index component was identified by HPLC.

The ECE was encapsulated to contain 200 mg per tablet. Compositional analysis of test products is presented in Table 1. The test group consumed 200 mg of ECE twice a day (400 mg day⁻¹) for 12 weeks and the control group consumed the placebo.

Table 1: Composition of test products provided per tablet

Components	Contents (mg)
Tests	
ECE	200.00
Dextrin	100.00
Crystallized cellulose	270.00
Silicon dioxide	6.00
Magnesium stearate	6.00
Coating with HPMC	18.00
Total	600.00
Placebos	
ECE	0.00
Dextrin	120.00
Crystallized cellulose	426.00
Silicon dioxide	3.00
Magnesium stearate	3.00
Caramel	30.00
Total	600.00

ECE: *Ecklonia cava* extract, HPMC: Hydroxypropyl methylcellulose

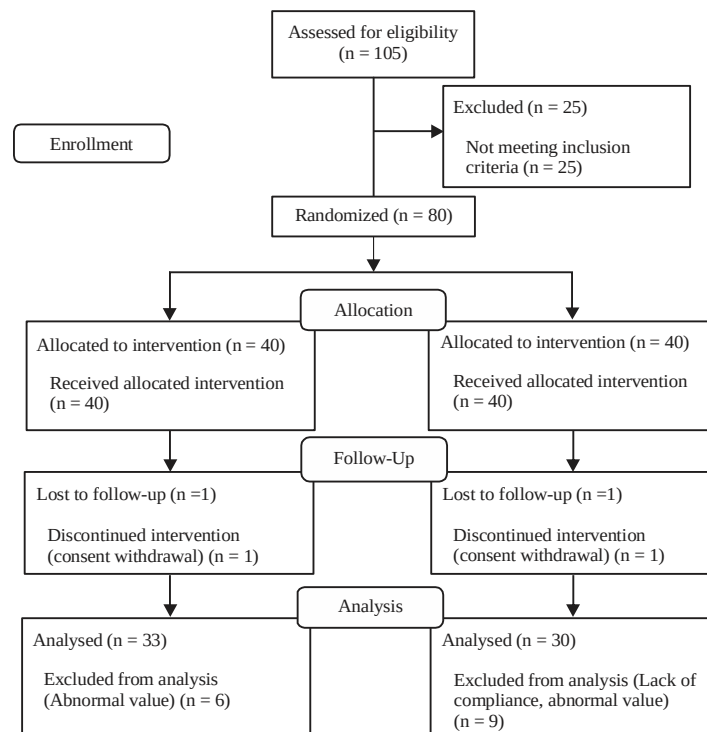


Fig. 1: CONSORT flow diagram

Biochemical measurements: The lipid levels were measured using a Hitachi 7600-110[®] analyzer (Hitachi High-Technologies Corporation, Tokyo, Japan). Hematology (CBC) and other laboratory tests (total protein, albumin, ALP, γ -GTP, AST, ALT, BUN, creatinine, glucose, creatine kinase and urinalysis) were measured using the Sysmex XE-5000[™] (Sysmex Corporation, Kobe, Japan) and ADVIA[®] 2400 chemistry system (SIEMENS, Munich, Germany), respectively. Venous blood samples were taken after a 12 h overnight fasting. All parameters were assessed the day of sampling.

Statistical analysis: For determining the sample size, a power of 80% with a 2-tailed α of 0.05 and equal sample size for both groups were assumed. We assumed mean differences of total cholesterol level in test and placebo group after 12 weeks to be 10.1 and 5.6 mg dL⁻¹, respectively and Standard Deviation (SD) of 7.2 mg dL⁻¹ at both groups. Therefore, a total of eighty subjects were needed, allowing a 20% dropout rate.

All data has been analyzed with the statistical software system SAS[®] version 9.2 (SAS Institute, USA). The differences among continuous measured data within a group were analyzed using a paired t-test. The differences of repeated measured data between groups was analyzed using a linear mixed-effects model. The data was presented as Mean $\pm$ SD. A difference was considered statistically significant when the probability value (p-value) was less than 0.05.

RESULTS

Subjects, demographics and safety assessments: Among the 105 volunteers screened, 80 participants who fulfilled the inclusion criteria were randomly assigned to an ECE group (n = 40) or a placebo group (n = 40). One subject from each group dropped out for a reason of consent withdrawal during the study. Among a total of 78 participants, who completed the study, 15 people were excluded from the data analysis due to a lack of pill compliance and abnormal blood lipid levels suggesting familial hypercholesterolemia. The study flow through the trial is depicted in Fig. 1.

The groups were well balanced. There were no significant differences in any of the baseline demographic data and anthropometric parameters between the ECE and placebo group (Table 2). All values of safety parameters including vital signs, ECG and blood laboratory tests (CBC, total protein, albumin, ALP, γ -GTP, AST, ALT, BUN, creatinine, glucose, creatine kinase and urinalysis) were within the reference range throughout the 12 week intervention period. There were no significant differences in any changes of the above values and anthropometric parameters in between the groups comparison.

There were only 10 cases of mild adverse events in 8 subjects (e.g., 1 degenerative osteoarthritis, 2 esophagitis, 1 wrist pain, 2 headache, 1 feeling of generalized swelling, 1 toothache, 1 non-specific pain and 1 implantation of teeth) that were not related to consumption of the test products.

Efficacy evaluations: A total of 80 participants were characterized by mild hypercholesterolemia at the time of enrollment (baseline total cholesterol: 213.21 $\pm$ 26.46 mg dL⁻¹, baseline LDL-cholesterol: 144.48 $\pm$ 23.78 mg dL⁻¹). There were no significant differences at the baseline levels of total and LDL-cholesterol between the ECE and placebo group (total cholesterol: p = 0.824, LDL-cholesterol: p = 0.508). After the 12 week intervention, total cholesterol level of the ECE group tended to decrease from the baseline and that of the placebo group tended to increase, so there was a statistically significant difference in changes of total cholesterol levels between the two groups (p = 0.039) (Table 3). The LDL-cholesterol level of the ECE group significantly decreased (p<0.001) and that of the placebo group did not change (p = 0.523). Therefore, there was a statistically significant difference in changes of LDL-cholesterol levels between the two groups (p = 0.030) (Table 3). The HDL-cholesterol level of the ECE group did not change (p = 0.316) and that of the placebo group significantly increased (p = 0.037), which resulted in a statistical difference in changes of HDL-cholesterol levels between the two groups (p = 0.021) (Table 3). Triglyceride and WHR levels of both

Table 2: Baseline demographic and anthropometric characteristics

Parameters	ECE group (n = 40)	Placebo group (n = 40)	Total (n = 80)	p-value ¹⁾
Age (years)	49.75 $\pm$ 10.03	49.83 $\pm$ 9.340	49.79 $\pm$ 9.630	0.973
Sex (M/F)	10/30	10/30	20/60	1.000 ²⁾
Height (cm)	161.49 $\pm$ 7.720	160.79 $\pm$ 8.650	161.14 $\pm$ 8.150	0.704
Weight (kg)	66.20 $\pm$ 10.55	66.33 $\pm$ 11.66	66.26 $\pm$ 11.04	0.959
BMI (kg m ⁻²)	25.30 $\pm$ 2.950	25.53 $\pm$ 2.970	25.41 $\pm$ 2.940	0.734
SBP (mm Hg)	121.55 $\pm$ 14.17	121.98 $\pm$ 12.10	121.76 $\pm$ 13.09	0.886
DBP (mm Hg)	74.85 $\pm$ 11.68	77.70 $\pm$ 10.41	76.28 $\pm$ 11.08	0.253
Pulse (BPM)	68.63 $\pm$ 8.600	68.45 $\pm$ 8.750	68.54 $\pm$ 8.620	0.928
WC (cm)	87.09 $\pm$ 8.150	88.44 $\pm$ 8.530	87.76 $\pm$ 8.310	0.470
HC (cm)	95.82 $\pm$ 5.320	95.50 $\pm$ 5.550	95.66 $\pm$ 5.400	0.795
WHR	0.91 $\pm$ 0.060	0.93 $\pm$ 0.060	0.92 $\pm$ 0.060	0.218

Data is presented as Mean $\pm$ SD, ¹⁾p-value was calculated with independent t-test, ²⁾p-value was calculated with Chi-square test, BMI: Body mass index, BPM: Beat per minute, DBP: Diastolic blood pressure, ECE: *Ecklonia cava* extract, F: Female, HC: Hip circumference, M: Male, SBP: Systolic blood pressure, WC: Waist circumference, WHR: Waist to hip ratio

Table 3: Effects of ECE supplementation on blood lipid levels

Parameters	ECE groups (n = 33)			Placebo groups (n = 30)			
	0 week	12 weeks	p-value ¹⁾	0 week	12 weeks	p-value ¹⁾	p-value ²⁾
Total cholesterol (mg dL ⁻¹)	207.39±23.10	201.58±20.72	0.164	202.03±17.41	208.50±23.20	0.129	0.039
LDL-cholesterol (mg dL ⁻¹)	141.42±19.81	125.52±21.98	<0.001	137.93±19.66	135.27±25.01	0.523	0.030
HDL-cholesterol (mg dL ⁻¹)	48.94±7.810	47.97±8.020	0.316	47.87±8.380	50.57±9.020	0.037	0.021
Triglyceride (mg dL ⁻¹)	122.91±50.43	143.21±69.80	0.076	131.40±69.09	142.00±68.37	0.306	0.523

Data is presented as Mean±S.D, ¹⁾p-value was calculated with paired t-test for within-treatment differences, ²⁾p-value was calculated with linear mixed effect model for between-treatment differences, ECE: *Ecklonia cava* extract, HDL: High density lipoprotein, LDL: Low density lipoprotein

Table 4: Effects of ECE supplementation on anthropometric parameters

Parameters	ECE groups (n = 33)			Placebo groups (n = 30)			
	0 week	12 weeks	p-value ¹⁾	0 week	12 weeks	p-value ¹⁾	p-value ²⁾
Height (cm)	161.52±8.290	161.33±8.360	0.312	160.45±8.820	160.37±8.980	0.495	0.636
Weight (kg)	66.33±10.41	66.48±10.28	0.573	65.64±12.29	65.66±12.78	0.954	0.725
BMI (kg m ⁻²)	25.35±2.920	25.48±2.930	0.233	25.31±2.600	25.33±2.800	0.860	0.469
Body fat mass (kg)	20.99±6.820	20.74±6.320	0.790	21.04±3.390	21.19±4.050	0.630	0.698
WC (cm)	87.35±7.610	86.82±7.270	0.144	87.84±7.670	87.58±7.330	0.634	0.681
HC (cm)	95.65±5.370	95.33±5.370	0.219	94.99±5.390	94.95±5.500	0.905	0.497
WHR	0.91±0.060	0.91±0.050	0.487	0.93±0.060	0.92±0.050	0.452	0.786

Data is presented as Mean±S.D, ¹⁾p-value was calculated with paired t-test for within-treatment differences, ²⁾p-value was calculated with linear mixed effect model for between-treatment differences, BMI: Body mass index, ECE: *Ecklonia cava* extract, HC: Hip circumference, WC: Waist circumference, WHR: Waist to hip ratio

groups did not change and there were no significant differences in changes of these parameters between the two groups (Table 3 and 4).

DISCUSSION

In this placebo-controlled trial for subjects with mild hypercholesterolemia, ECE supplementation at a dosage of 400 mg day⁻¹ for 12 weeks significantly decreased serum total and LDL-cholesterol levels as compared to the placebo. The HDL-cholesterol level of the placebo group significantly increased as compared to that of the ECE group. No corresponding changes were seen for triglyceride and WHR levels between the two groups. The ECE were considered safe to ingest because there were no adverse drug reactions which were related to consumption of test products and the parameters including vital signs, physical examination, ECG and blood laboratory tests were maintained within the reference range.

Our results that ECE supplementation significantly lowered total and LDL-cholesterol levels (Table 3) are in accord with the previous studies. Shin *et al.* (2012) reported that polyphenols extracted from EC decreased total cholesterol by 9.2% and LDL-cholesterol by 14.2% in overweight human subjects (Shin *et al.*, 2012). The reduction of 2.8% in total cholesterol and the 11.1% for LDL-cholesterol at 12 weeks, obtained with 400 mg day⁻¹ of ECE in this study were lower than those obtained by Shin *et al.* (2012) at a dosage of 144 mg day⁻¹ from a similar baseline levels, which can be attributed to the different test products used and highlights the need to express the concentration of active substances of these extracts according to quality standards. Yoon *et al.* (2008) reported that extract of *Ecklonia stolonifera* (ES) and its phlorotannin constituents, eckols and dieckols, reduced total and LDL-cholesterol levels in hyperlipidemic rats.

These results suggest that ingestion of ECE may improve blood lipid profiles by modulation of lipid metabolism and contribute to reduction of cardiovascular risks by decreasing LDL-cholesterol levels in patients with hypercholesterolemia because LDL-cholesterol levels are closely related to atherosclerotic cardiovascular risks (Kannel *et al.*, 1979; Gordon *et al.*, 1981; Lipid Research Clinics Programme, 1984).

Mechanisms explaining the anti-dyslipidemic effects of polyphenols or phlorotannins of EC are not clear. Yeo *et al.* (2012) showed that both polyphenols and dieckol isolated from EC inhibited the activity of HMG-CoA reductase. Suppression of fat-induced hepatic damage by anti-oxidant, anti-inflammatory and hepatoprotective activities of EC polyphenols might play a role in improving blood lipids (Shin *et al.*, 2012), based on a report that phlorotannin derived from brown algae protected Hep G2 cells against the cytotoxic effects of tacrine (Kim *et al.*, 2005) and reports regarding that an impaired anti-oxidant system, reduced antioxidant defense potentials and increased peroxidation were found in steatotic liver tissues of hyperlipidemic animals (Arhan *et al.*, 2009).

There are contradictory reports regarding the effects of EC on blood HDL-cholesterol. In our results, HDL-cholesterol level changed little in the ECE group while this level increased in the placebo group, which resulted in a statistically significant difference between the two groups after the 12 week intervention (Table 3). Yeo *et al.* (2012) also reported both polyphenols and dieckols isolated from EC did not increase serum HDL-cholesterol levels but significantly lowered total-cholesterol, LDL-cholesterol and triglyceride levels in High-Fat Diet (HFD)-fed mice. On the other hand, according to the other studies mentioned above, both extracts

from EC (Shin *et al.*, 2012) and ES (Yoon *et al.*, 2008) not only increased HDL-cholesterol levels but also lowered total and LDL-cholesterol levels.

There is a general consensus about the “HDL hypothesis” that HDL-cholesterol protects against atherosclerosis (Barr *et al.*, 1951; Gordon *et al.*, 1977; Vergeer *et al.*, 2010). However, it has been increasingly questioned whether HDL-cholesterol would indeed reduce the risk of atherosclerosis, as some studies showed that genetically low (Frikke-Schmidt, 2010) or high HDL-cholesterol (Johannsen *et al.*, 2009; Voight *et al.*, 2012) did not correspond to expected outcomes in coronary heart disease risks and drugs that raised HDL-cholesterol failed to reduce CVD events (Barter *et al.*, 2007; The ACCORD Study Group, 2010) or atherosclerosis (Nissen *et al.*, 2007). It was recently suggested that HDL quantity (e.g., HDL particle or apolipoprotein A1) or HDL quality (e.g., particle size, subclass distribution) (Rosenson *et al.*, 2011) or various measures of HDL functionality (De Goma *et al.*, 2008; Angeloni *et al.*, 2013) may be better markers than plasma HDL-cholesterol concentration itself.

Therefore, based on the studies discussed above, the fact that this study did not demonstrate an increase in HDL-cholesterol concentration does not mean that we failed to demonstrate an improvement in an atheroprotective marker, although we only examined the overall HDL-cholesterol concentration and did not examine HDL quantity or quality.

There were other discrepancies in anthropometric parameters. They were not improved in our study (Table 4) while Shin *et al.* (2012) reported that polyphenols extracted from EC improved anthropometric parameters such as BMI, waist circumference and WHR as well as lipid profiles in overweight subjects and Yeo *et al.* (2012) showed that both polyphenols and dieckols isolated from EC significantly inhibited body weight gain compared to only HFD-fed mice.

Mechanisms explaining the anti-obesity effects of polyphenols or phlorotannins of EC are also not clear. Yeo *et al.* (2012) reported that both polyphenols and dieckols isolated from EC inhibited 3T3-L1 adipocyte differentiation, Kong *et al.* (2010) showed that a compound isolated from EC inhibited lipid accumulation and adipocyte differentiation by the down-regulation of the genes involved in lipogenesis and adipogenesis and Ko *et al.* (2013) showed that dieckol, a phlorotannin isolated from EC, inhibited adipogenesis through AMP-activated protein kinase (AMPK) activation in 3T3-L1 cells.

This randomized double-blind, placebo-controlled trial showed that the ECE had the efficacy on the blood lipid profile. Moreover, these results on the lipid profile were similar to those obtained with other dietary supplements (Ros, 2010; Gylling *et al.*, 2014), some of which have been shown not only to improve the lipid profile but to reduce cardiovascular morbidity and mortality in the context of a Mediterranean diet (Ros, 2010; Estruch *et al.*, 2013). However,

as discussed above, contradictory reports exist about the effects of polyphenols or phlorotannins of EC on blood lipid profiles and anthropometric parameters. These discrepancies may be explained by differences in several factors, such as EC extract production process and study design including dosage and participant characteristics.

This study has some limitations. We did not thoroughly investigate the participants' daily diets and physical activities and did not prohibit the participants from consuming seaweed. However, we made an effort to have the participants maintain their usual diet and physical activities during the 12 week intervention period and asked them to restrict their seaweed consumption to the utmost.

It is now widely accepted as a scientific fact that lifestyle modification with emphasis on healthy diet patterns is crucial in the management of hypercholesterolemia. In this trial, ECE supplementation improved blood lipid profiles through decreasing total cholesterol and LDL-cholesterol levels. This result suggests that ECE supplementation has the potential to reduce cardiovascular risks and may be effective in the treatment of atherosclerotic CVD in a safe manner, although it is necessary to perform more studies with clinical (hard) end-points to establish its long-term effectiveness and safety.

ACKNOWLEDGMENTS

We gratefully acknowledge the contribution of the subjects, research coordinators and all the staff who supported the administration of the trial. We thank BotaMedi Inc. (Jeju, South Korea) for providing test products. This study was supported by the Korea Institute for Advancement of Technology (KIAT) and the Jeju Institute for Regional Program Evaluation through the Leading Industry Development for Economic Region grant funded by the Ministry of Trade, Industry and Energy (MOTIE).

REFERENCES

- Abd, T.T. and T.A. Jacobson, 2011. Statin-induced myopathy: A review and update. *Exp. Opin. Drug Saf.*, 10: 373-387.
- Ahn, G.N., K.N. Kim, S.H. Cha, C.B. Song and J. Lee *et al.*, 2007. Antioxidant activities of phlorotannin purified from *Ecklonia cava* on free radical scavenging using ESR and H₂O₂-mediated DNA damage. *Eur. Food Res. Technol.*, 226: 71-79.
- Ahn, M.J., K.D. Yoon, S.Y. Min, J.S. Lee and J.H. Kim *et al.*, 2004. Inhibition of HIV-1 reverse transcriptase and protease by phlorotannin from the brown alga *Ecklonia cava*. *Biol. Pharm. Bull.*, 27: 544-547.
- Angeloni, E., F. Paneni, U. Landmesser, U. Benedetto and G. Melina *et al.*, 2013. Lack of protective role of HDL-C in patients with coronary artery disease undergoing elective coronary artery bypass grafting. *Eur. Heart J.*, 34: 3557-3562.

- Arhan, M., H.S. Ozturk, N. Turhan, B. Aytac, M.C. Guven, E. Olcay and I. Durak, 2009. Hepatic oxidant/antioxidant status in cholesterol-fed rabbits: Effects of garlic extract. *Hepatol. Res.*, 39: 70-77.
- Barr, D.P., E.M. Russ and H.A. Eder, 1951. Protein-lipid relationships in human plasma. II. In atherosclerosis and related conditions. *Am. J. Med.*, 11: 480-493.
- Barter, P.J., M. Caulfield, M. Eriksson, S.M. Grundy and J.J. Kastelein *et al.*, 2007. Effects of torcetrapib in patients at high risk for coronary events. *New Engl. J. Med.*, 357: 2109-2122.
- Brown, A.L., J. Lane, J. Coverly, J. Stocks and S. Jackson *et al.*, 2009. Effects of dietary supplementation with the green tea polyphenol epigallocatechin-3-gallate on insulin resistance and associated metabolic risk factors: Randomized controlled trial. *Br. J. Nutr.*, 101: 886-894.
- Davison, K., A.M. Coates, J.D. Buckley and P.R.C. Howe, 2008. Effect of cocoa flavanols and exercise on cardiometabolic risk factors in overweight and obese subjects. *Int. J. Obesity*, 32: 1289-1296.
- De Goma, E.M., R.L. de Goma and D.J. Rader, 2008. Beyond high-density lipoprotein cholesterol levels: Evaluating high-density lipoprotein function as influenced by novel therapeutic approaches. *J. Am. College Cardiol.*, 51: 2199-2211.
- Eckel, R.H., J.M. Jakicic, J.D. Ard, J.M. de Jesus and N.H. Miller *et al.*, 2014. 2013 AHA/ACC guideline on lifestyle management to reduce cardiovascular risk: A report of the American college of cardiology/American heart association task force on practice guidelines. *Circulation*, 129: S76-S99.
- Edwards, R.L., T. Lyon, S.E. Litwin, A. Rabovsky, J.D. Symons and T. Jalili, 2007. Quercetin reduces blood pressure in hypertensive subjects. *J. Nutr.*, 137: 2405-2411.
- Estruch, R., E. Ros, J. Salas-Salvado, M.I. Covas and D. Corella *et al.*, 2013. Primary prevention of cardiovascular disease with a Mediterranean diet. *New Engl. J. Med.*, 368: 1279-1290.
- Frikke-Schmidt, R., 2010. Genetic variation in the *ABCA1* gene, HDL cholesterol and risk of ischemic heart disease in the general population. *Atherosclerosis*, 208: 305-316.
- Gordon, T., W.P. Castelli, M.C. Hjortland, W.B. Kannel and T.R. Dawber, 1977. High density lipoprotein as a protective factor against coronary heart disease: The Framingham study. *Am. J. Med.*, 62: 707-714.
- Gordon, T., W.B. Kannel, W.P. Castelli and T.R. Dawber, 1981. Lipoproteins, cardiovascular disease and death: The framingham study. *Arch. Internal Med.*, 141: 1128-1131.
- Grassi, D., S. Necozione, C. Lippi, G. Croce and L. Valeri *et al.*, 2005. Cocoa reduces blood pressure and insulin resistance and improves endothelium-dependent vasodilation in hypertensives. *Hypertension*, 46: 398-405.
- Gylling, H., J. Plat, S. Turley, H.N. Ginsberg and L. Ellegard *et al.*, 2014. Plant sterols and plant stanols in the management of dyslipidaemia and prevention of cardiovascular disease. *Atherosclerosis*, 232: 346-360.
- Harada, H. and Y. Kamei, 1997. Selective cytotoxicity of marine algae extracts to several human leukemic cell lines. *Cytotechnology*, 25: 213-219.
- Heart Protection Study Collaborative Group, 2002. MRC/BHF heart protection study of cholesterol lowering with simvastatin in 20,536 high-risk individuals: A randomised placebo-controlled trial. *Lancet*, 360: 7-22.
- Heo, S.J., E.J. Park, K.W. Lee and Y.J. Jeon, 2005. Antioxidant activities of enzymatic extracts from brown seaweeds. *Bioresour. Technol.*, 96: 1613-1623.
- Hyun, J.A., S.K. Hyun, H.R. Kim and J.S. Choi, 2006. Angiotensin-converting enzyme I inhibitory activity of phlorotannins from *Ecklonia stolonifera*. *Fish. Sci.*, 72: 1292-1299.
- Johannsen, T.H., P.R. Kamstrup, R.V. Andersen, G.B. Jensen, H. Sillesen, A. Tybjaerg-Hansen and B.G. Nordestgaard, 2009. Hepatic lipase, genetically elevated high-density lipoprotein and risk of ischemic cardiovascular disease. *J. Clin. Endocrinol. Metab.*, 94: 1264-1273.
- Joy, T.R. and R.A. Hegele, 2009. Narrative review: Statin-related myopathy. *Ann. Internal Med.*, 150: 858-868.
- Kang, C., Y.B. Jin, H. Lee, M. Cha and E.T. Sohn *et al.*, 2010. Brown alga *Ecklonia cava* attenuates type 1 diabetes by activating AMPK and Akt signaling pathways. *Food Chem. Toxicol.*, 48: 509-516.
- Kannel, W.B., W.P. Castelli and T. Gordon, 1979. Cholesterol in the prediction of atherosclerotic disease. New perspectives based on the Framingham study. *Ann. Internal Med.*, 90: 85-91.
- Kim, M.M., Q.V. Ta, E. Mendis, N. Rajapakse and W.K. Jung *et al.*, 2006. Phlorotannin in *Ecklonia cava* extract inhibit matrix metalloproteinase activity. *Lift Sci.*, 79: 1436-1443.
- Kim, Y.C., R.B. An, N.Y. Yoon, T.J. Nam and J.S. Choi, 2005. Hepatoprotective constituents of the edible brown alga *Ecklonia stolonifera* on tacrine-induced cytotoxicity in hep G2 cells. *Arch. Pharm. Res.*, 28: 1376-1380.
- Knekt, P., R. Jarvinen, A. Reunanen and J. Maatela, 1996. Flavonoid intake and coronary mortality in Finland: A cohort study. *Br. Med. J.*, 312: 478-481.
- Ko, S.C., M. Lee, J.H. Lee, S.H. Lee, Y. Lim and Y.J. Jeon, 2013. Dieckol, a phlorotannin isolated from a brown seaweed, *Ecklonia cava*, inhibits adipogenesis through AMP-activated protein kinase (AMPK) activation in 3T3-L1 preadipocytes. *Environ. Toxicol. Pharmacol.*, 36: 1253-1260.
- Kong, C.S., J.A. Kim, B.N. Ahn, V.T. Sang and N.Y. Yoon *et al.*, 2010. 1-(3',5'-dihydroxyphenoxy)-7-(2,4,6-trihydroxyphenoxy)-2,4,9-trihydroxydibenzo-1,4-dioxin inhibits adipocyte differentiation of 3T3-L1 fibroblasts. *Mar. Biotechnol.*, 12: 299-307.

- Lipid Research Clinics Programme, 1984. The lipid research clinics coronary primary prevention trial results. II. The relationship of reduction in incidence of coronary heart disease to cholesterol lowering. J. Am. Med. Assoc., 25: 365-374.
- Llorente-Mirandes, T., M.J. Ruiz-Chancho, M. Barbero, R. Rubio and J.F. Lopez-Sanchez, 2011. Determination of water-soluble arsenic compounds in commercial edible seaweed by LC-ICPMS. J. Agric. Food Chem., 59: 12963-12968.
- Martinez, J.H.I. and H.G.T. Castaneda, 2013. Preparation and chromatographic analysis of phlorotannin. J. Chromatogr. Sci., 51: 825-838.
- Nagao, T., T. Hase and I. Tokimitsu, 2007. A green tea extract high in catechins reduces body fat and cardiovascular risks in humans. Obesity, 15: 1473-1483.
- National Cholesterol Education Program, 2001. Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation and treatment of high blood cholesterol in adults (Adult treatment panel III). JAMA., 285: 2486-2497.
- Nissen, S.E., E.M. Tuzcu, P. Schoenhagen, B.G. Brown and P. Ganz *et al.*, 2004. Effect of intensive compared with moderate lipid-lowering therapy on progression of coronary atherosclerosis: A randomized controlled trial. J. Am. Med. Assoc., 291: 1071-1080.
- Nissen, S.E., J.C. Tardif, S.J. Nicholls, J.H. Revkin and C.L. Shear *et al.*, 2007. Effect of torcetrapib on the progression of coronary atherosclerosis. New Engl. J. Med., 356: 1304-1316.
- Ros, E., 2010. Health benefits of nut consumption. Nutrients, 2: 652-682.
- Rosenson, R.S., H.B. Jr. Brewer, M.J. Chapman, S. Fazio and M.M. Hussain *et al.*, 2011. HDL measures, particle heterogeneity, proposed nomenclature and relation to atherosclerotic cardiovascular events. Clin. Chem., 57: 392-410.
- Shibata, T., K. Ishimaru, S. Kawaguchi, H. Yoshikawa and Y. Hama, 2008. Antioxidant activities of phlorotannin isolated from Japanese Laminariaceae. J. Applied Phycol., 20: 705-711.
- Shim, S.Y., L.Q. To, S.H. Lee and S.K. Kim, 2009. *Ecklonia cava* extract suppresses the high-affinity IgE receptor, FcεRI expression. Food Chem. Toxicol., 47: 555-560.
- Shin, H.C., S.H. Kim, Y. Park, B.H. Lee and H.J. Hwang, 2012. Effects of 12-week oral supplementation of *Ecklonia cava* polyphenols on anthropometric and blood lipid parameters in overweight korean individuals: A double-blind randomized clinical trial. Phytother. Res., 26: 363-368.
- The ACCORD Study Group, 2010. Effects of combination lipid therapy in type 2 diabetes mellitus. New Engl. J. Med., 362: 1563-1574.
- Vergeer, M., A.G. Holleboom, J.J.P. Kastelein and J.A. Kuivenhoven, 2010. The HDL hypothesis: Does high-density lipoprotein protect from atherosclerosis? J. Lipid Res., 51: 2058-2073.
- Voight, B.F., G.M. Peloso, M. Orho-Melander, R. Frikke-Schmidt and M. Barbalic *et al.*, 2012. Plasma HDL cholesterol and risk of myocardial infarction: A mendelian randomisation study. Lancet, 380: 572-580.
- Wang, T., R. Jonsdottir, H. Liu, L. Gu, H.G. Kristinsson, S. Raghavan and G. Olafsdottir, 2012. Antioxidant capacities of phlorotannins extracted from the brown algae *Fucus vesiculosus*. J. Agric. Food Chem., 60: 5874-5883.
- Yeo, A.R., J.L. Lee, I.H. Tae, S.R. Park and Y.H. Cho *et al.*, 2012. Anti-hyperlipidemic effect of polyphenol extract (Seapolynol™) and dieckol isolated from *Ecklonia cava* in *in vivo* and *in vitro* models. Preventive Nutr. Food Sci., 17: 1-7.
- Yoon, N.Y., H.R. Kim, H.Y. Chung and J.S. Choi, 2008. Anti-hyperlipidemic effect of an edible brown algae, *Ecklonia stolonifera* and its constituents on poloxamer 407-induced hyperlipidemic and cholesterol-fed rats. Arch. Pharm. Res., 31: 1564-1571.