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Insulinotrophic and hypolipidemic effects of *Ecklonia cava* in streptozotocin-induced diabetic mice

Mi-Ja Kim¹, Hye Kyung Kim^{2*}¹Department of Food and Biotechnology, Hanseo University, Seosan 356–706, Republic of Korea²Department of Pharmacology, school of Medicine, Kyunghee University, Seoul 130–701, Republic of Korea

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ABSTRACT

Objective: To explore the anti-diabetic activity of *Ecklonia cava* (EC) in streptozotocin (STZ)-induced diabetic mice. **Methods:** Diabetes was induced by a single intraperitoneal injection of STZ (90 mg/kg). Normal and diabetic mice were treated with 0%, 3%, and 5% EC diet for 4 weeks. Serum glucose and insulin concentrations, serum lipid profile, oral glucose tolerance test, and liver and pancreatic β -cell histopathological observations were performed. In addition, *in vitro* glucose-induced insulin secretion was determined using pancreatic β -islet cells. **Results:** EC supplementation significantly and dose-dependently decreased serum glucose concentration, and improved glucose homeostasis in diabetic mice by preventing loss of β -cell mass resulting in increase of insulin secretion. The triglyceride and total cholesterol concentrations in the serum and liver were markedly reduced by EC treatment in STZ-diabetic mice. Moreover, LDL-, and HDL-cholesterol levels were ameliorated in EC supplemented diabetic mice. Liver steatosis induced by STZ was ameliorated by EC supplementation. Furthermore, *in vitro* insulinotrophic effect of EC extract was observed in pancreatic β -islets. **Conclusions:** This study demonstrated that EC is a potent and efficacious hypoglycemic and hypolipidemic agent, and prevents the loss of β -cell mass resulting in increase of insulin secretory capacity.

1. Introduction

Diabetes is a common metabolic disorder characterized by hyperglycemia due to absolute or relative deficiency of insulin secretion from pancreatic cells[1]. Once hyperglycemia becomes apparent, β -cell function progressively deteriorates: glucose-induced insulin secretion becomes further impaired and degranulation of β -cells becomes evident, often accompanied by a decrease in the number of β -cells[2]. The majority of patients with obesity causing insulin resistance are not diabetic, as their capacity for β -cell compensation is maintained but, 15%–20% of these individuals become diabetic, when

the β -cells lose their compensatory ability[3]. Therefore, one approach to preventing and treating diabetes could be through the enhancement of β -cell mass. Insulin deficiency stimulates lipolysis in the adipose tissue, and gives rise to hyperlipidemia and fatty liver[4]. Accordingly, although diabetes is characterized as a disease of carbohydrate metabolism, abnormalities of lipid and lipoprotein metabolism are commonly observed[5].

Marine algae have begun to attract attention as rich sources of diverse bioactive compounds with great pharmaceutical and biomedical potential. *Ecklonia cava* (EC) is an abundant brown algae used as a seasoned vegetable in the coastal regions of Asian countries. It has been reported that EC extract have numerous biological activities, including anti-oxidative, radical scavenging, immunomodulatory, and anti-mutagenic activities[6–10]. Recently, polyphenol from EC has been reported to inhibit α -amylase and α -glycosidase activities resulting in reduction of postprandial hyperglycemia in diabetic

*: Corresponding author: Dr. HK Kim, Professor, Hanseo University, Seosan 356–706, Republic of Korea.
Tel : +82416601454
Fax : +82416601402
E-mail: hkkim111@dreamwiz.com

mice^[11]. In present study, we extended the work to examine the possible anti-diabetic mechanism of EC in STZ-induced diabetic mice as a model system of type-1 diabetes, an example of elevated blood lipids and insulin deficiency.

2. Materials and methods

2.1. Preparation of EC powder and extract

EC was obtained from a local market in Seosan, Korea. Fresh EC was washed, dried in the shade, and ground into powder. The EC powder was used for the *in vivo* experiment and EC extract was used for the *in vitro* experiment. The dried powder was extracted three times with ten volumes of methanol at room temperature for 24 h. The combined extracts were centrifuged, filtered, concentrated under vacuum, lyophilized, and subsequently used for the experiment. The yield after vacuum evaporation was 12.9%.

2.2. Animals and diets

Male ICR mice (8 wks old, Joong Ang Lab Animal Co., Korea) were housed in plastic cages under temperature (24±2) °C and light (12-h light/dark cycle) controlled conditions with constant humidity (55±5)%. All animals had free access to standard rodent pellet food, except when fasted before experiments. The study has been approved by Animal Ethics Committee of Kyunghee University, and carried out along the Korea National Institutes of Health Guidelines on the care and use of laboratory animals. The mice were randomly divided into 6 groups ($n = 10$); normal control (NC), normal mice fed 3% EC powder (NE3), normal mice fed 5% EC (NE5), diabetic control (DC), diabetic mice fed 3% EC (DE3), and 5% EC (DE5).

Diabetes was induced by a single intraperitoneal injection of STZ (Sigma, St. Louis, USA, 90 mg/kg in citrate buffer, pH 4.5). Normal groups received the buffer only. Tail bleeds were performed 24 h post-injection, and animals with a blood glucose concentration above 300 mg/dL were considered to be diabetic and used in this study.

2.3. Oral glucose tolerance test and biochemical analysis

At the end of 4 wks of experimental period, mice were fasted overnight and orally administered with glucose (1.5 g/kg). Blood samples were collected from the tail at various time points (0–90 min) after glucose loading, and blood glucose levels were measured by One Touch Basic Glucose Measurement System (Life Scan Inc., USA). Mice were killed by decapitation immediately 90 min after blood samples were taken. Serum concentrations of glucose, aspartate aminotransferase (AST), alanine aminotransferase

(ALT), total cholesterol (TC), HDL-cholesterol (HDL-C), and triglyceride (TG) were measured using commercially available kits (Youngdong Pharmaceutical Co., Korea). Serum LDL-cholesterol (LDL-C) levels were calculated by Friedwald equation^[12]. Serum insulin concentrations were determined by using radioimmunoassay kit (Boehringer Mannheim, Germany). The hepatic lipids were extracted using the procedure developed by Folch *et al*^[13], and the TC and TG concentrations were measured using same kits as serum.

2.4. Histological analysis

Pancreatic and hepatic tissue fragments were fixed in 10% neutralized formalin solution for 24 h. After fixing, the tissues were dehydrated, embedded in paraffin, and sectioned with 5 μ m thickness (Leica, Wetzlar, Germany). Histological staining of β -cells using aldehyde fuchsin staining, and hematoxylin and eosin (H&E) staining for hepatic tissue were performed.

2.5. In vitro insulin secretion

Pancreatic islet cells were isolated from male mice by collagenase digestion^[14]. Twenty islets were pre-incubated in Krebs-Ringer Bicarbonate (KRB) buffer, pH 7.4, supplemented with serum albumin (3 mg/mL) and glucose (3 mM) for 30 min at 37 °C under humidified atmosphere of 5% CO₂ in air. The islets were treated with various concentrations of EC extract in 16 mM glucose KRB buffer for 60 min at 37 °C. Insulin concentration in each medium was determined using an ELISA procedure (Boehringer Mannheim Diagnostics, Germany).

2.6. Statistical analysis

Data were expressed as Mean±SEM. One-way ANOVA was used to determine treatment effects. Differences among means were inspected using Duncan's multiple range test and were considered to be significant at $P < 0.05$.

3. Results

3.1. Hypoglycemic effect

Induction of diabetes caused significant weight loss resulting in negative body weight gain in diabetic mice (Table 1). Interestingly, administration of EC significantly and dose-dependently decreased the weight gain in normal mice but prevents the weight loss in diabetic mice. Initial blood glucose levels were 3.6-fold elevated in STZ-diabetic mice (DC) compared with normal control (NC), and EC

supplement decreased hyperglycemia in a dose-dependent manner. Accordingly, administration of EC increased serum insulin levels of diabetic mice in a dose-dependent manner. EC supplementation at 5% of the diet for 4 wks elevated the serum insulin concentration to 86% of the normal level. Normal mice were not affected in serum glucose and insulin levels by EC supplement.

The results of the light microscopy investigation demonstrated that the pancreas of the normal mice showed normal pancreatic architecture while diabetic mice revealed relatively small, atrophied probably due to the reduction in the number of the cells, lightly stained, ambiguity of their verges, and degranulation of cells (Figure 1). EC supplemented diabetic mice exhibited obvious amelioration in histological signs. Strong staining and increased size of islets displaying less degenerative changes compared with DC group.

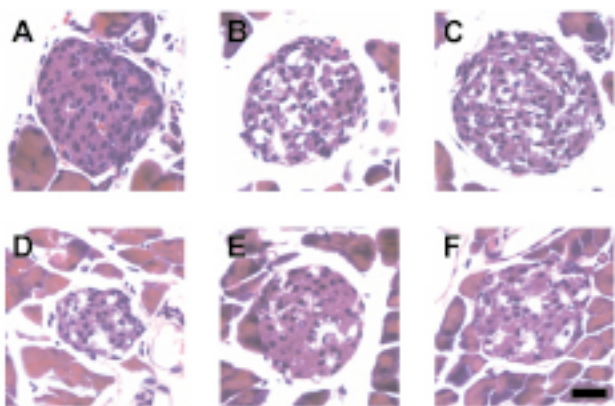


Figure 1. Images of histologically-stained β -cells in pancreas. Pancreases from normal control (A), normal mice treated with 3% EC (B), normal mice treated with 5% EC (C), STZ-diabetic control (D), diabetic mice treated with 3% EC (E), or diabetic mice treated with 5% EC (F) were stained with aldehyde fuchsin staining. Scale bar indicates 100 μ m.

To assess glucose homeostasis and insulin sensitivity in EC supplemented mice, we next performed OGTT. As shown in Figure 2, glucose load in NC produced rapid increase in blood glucose levels from 113 ± 4 to 254 ± 15 mg/dL at 30 min and returned to baseline values within 90 min. STZ-induced diabetic mice demonstrated basal hyperglycemia (394 ± 14 mg/dL) which remained above 400 mg/dL during all time points determined. The peak increase in serum glucose concentrations in diabetic mice was observed after 60 min of glucose treatment, while that of normal mice observed after 30 min, indicating delayed glucose homeostasis in diabetic mice. EC exerted no significant effect on the basal glucose or OGTT of normal mice. In contrast, 3% and 5% of EC supplementation in diabetic mice resulted in a significant dose-dependent reduction in fasting blood glucose levels combined with a significant improvement in glucose tolerance showing similar patterns as normal control group.

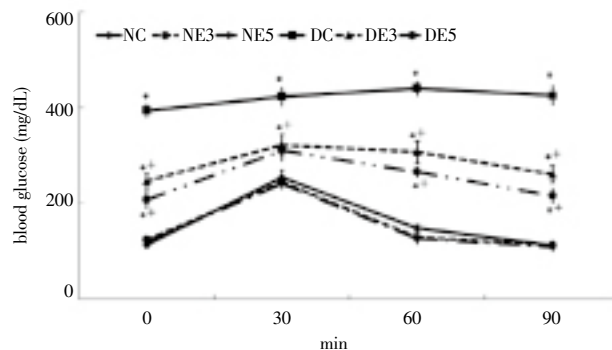


Figure 2. Effect of EC on oral glucose tolerance test.

Normal control mice (NC); normal mice supplemented with 3% EC (NE3); normal mice supplemented with 5% EC (NE5); STZ-diabetic control mice (DC); diabetic mice supplemented with 3% EC (DE3); diabetic mice supplemented with 5% EC (DE5).

* $P < 0.05$ vs. NC, † $P < 0.05$ vs. DC.

3.2. Hypolipidemic effect

TG, TC, and LDL-C concentrations in the serum were significantly higher in STZ-diabetic mice than normal control mice (Table 2). The supplementation of EC dose-dependently suppressed these parameters in both normal and diabetic mice. The serum TG, TC, and LDL-C levels of DE5 group were decreased by 72%, 53%, and 78%, respectively. HDL-C concentrations of DC were significantly lower than NC, and EC supplementation dose-dependently increased the HDL-C levels exhibiting 1.8-fold increase in DE5 group compared with DC group. EC treatments fail to affect HDL-C concentrations in normal mice.

The activities of liver function markers, serum AST and ALT concentrations in DC were significantly increased by 1.7- and 2.1-fold, respectively, compared with those of NC group (Table 2). EC supplementation dose-dependently reduced these marker enzyme activities in STZ-diabetic mice resulting in normal levels in DE5 group. Elevated liver TG and cholesterol levels in DC were dose-dependently decreased by EC administration resulting in normal levels of these parameters.

The results of the histological investigation of the liver are shown in Figure 3. DC group showed the accumulation of hepatic lipid droplets, and severe steatosis compare to NC group which exhibited normal hepatic histology. The clear vacuoles would have contained lipid in the living cells, however the histological fixation caused it to be dissolved and hence only empty spaces remain. In the EC supplemented groups, reduced lipid droplets were observed in a dose-dependent manner showing similar morphological liver condition to that of the normal group.

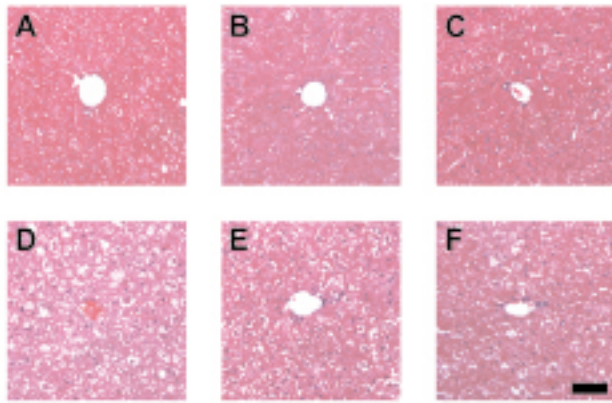


Figure 3. Effect of EC on hepatic tissue morphology.

Representative picture of H & E stained sections of liver tissue from normal control (A), normal mice treated with 3% EC (B), normal mice treated with 5% EC (C), STZ-diabetic control (D), diabetic mice treated with 3% EC (E), or diabetic mice treated with 5% EC (F). Scale bar indicates 50 μ m.

3.3. *In vitro* glucose-induced insulin secretion in pancreatic islets

To elucidate the mechanism of anti-diabetic effect of EC, the glucose-induced insulin secretion was examined in hyperglycemic condition (16 mM) using pancreatic β -islet cells. The data in Table 3 shows that insulin secretion increased with increasing concentrations of EC extract ($P < 0.05$). The EC-boosted glucose-induced insulin secretion peaked at 50 μ g/mL of EC extract in the medium, and declined significantly at EC concentrations higher than 50 μ g/mL.

4. Discussion

STZ-induced diabetes in adult animals has been used as a model for insulin-dependent diabetes and hypercholesterolemia, and has been shown to be sensitive

Table 1

Effects of EC on body weight, blood glucose and insulin.

Group	Weight gain (g/4wk)	Serum glucose(mg/dL)	Serum insulin(ng/mL)
NC	1.70 \pm 0.10	108.00 \pm 2.00	1.28 \pm 0.03
NE3	-2.60 \pm 0.10 [†]	106.00 \pm 2.00	1.25 \pm 0.03
NE5	-3.20 \pm 0.10 [†]	102.00 \pm 4.00	1.30 \pm 0.01
DC	-5.70 \pm 0.10 [*]	394.00 \pm 8.00 [*]	0.50 \pm 0.01 [*]
DE3	-1.60 \pm 0.20 ^{*†}	263.00 \pm 9.00 ^{*†}	0.86 \pm 0.03 ^{*†}
DE5	-1.90 \pm 0.20 ^{*†}	198.00 \pm 10.00 ^{*†}	1.09 \pm 0.02 ^{*†}

NC: normal control mice; NE3: normal mice supplied with 3% EC; NE5: normal mice fed 5% EC; DC: STZ-diabetic mice; DE: diabetic mice fed 3% EC; DE5: diabetic mice fed 5% EC.

* $P < 0.05$ vs. NC, [†] $P < 0.05$ vs. DC.

Table 2

Effects of EC on serum lipid profiles in STZ-diabetic mice

Group	TG (mg/dL)	TC(mg/dL)	LDL-C(mg/dL)	HDL-C(mg/dL)	AST(U/mL)	ALT(U/mL)	TG (mg/g)	Chol (mg/g)
NC	92.2 \pm 1.7	151.4 \pm 5.4	40.3 \pm 5.4	92.6 \pm 2.5	77.3 \pm 8.4	36.0 \pm 3.2	20.1 \pm 2.0	5.6 \pm 0.5
NE3	87.8 \pm 4.0	133.6 \pm 3.6 [†]	26.3 \pm 3.6 [†]	89.9 \pm 2.7	71.0 \pm 3.9	34.1 \pm 3.9	19.3 \pm 3.4	5.0 \pm 0.3
NE5	80.4 \pm 3.8 [†]	121.0 \pm 3.1 [†]	24.2 \pm 3.1 [†]	86.7 \pm 7.5	64.8 \pm 6.0	28.5 \pm 5.4	17.5 \pm 2.7	4.5 \pm 0.3 [†]
DC	244.6 \pm 4.5 [*]	279.0 \pm 6.5 [*]	188.3 \pm 6.5 [*]	41.7 \pm 4.7 [*]	133.5 \pm 8.4 [*]	76.0 \pm 5.7 [*]	35.4 \pm 4.4 [*]	9.1 \pm 1.3 [*]
DE3	81.3 \pm 2.0 ^{*†}	149.1 \pm 3.1 [†]	68.4 \pm 3.1 ^{*†}	64.4 \pm 0.8 ^{*†}	92.9 \pm 6.0 ^{*†}	52.9 \pm 6.2 ^{*†}	20.7 \pm 4.1 [†]	6.8 \pm 0.6 [†]
DE5	68.2 \pm 1.8 ^{*†}	130.4 \pm 5.4 ^{*†}	40.6 \pm 5.4 [†]	76.1 \pm 0.3 ^{*†}	80.4 \pm 5.0 [†]	37.9 \pm 7.7 [†]	17.9 \pm 2.2 [†]	5.1 \pm 0.5 [†]

NC: normal control mice; NE3: normal mice supplied with 3% EC; NE5: normal mice fed 5% EC; DC: STZ-diabetic mice; DE: diabetic mice fed 3% EC; DE5: diabetic mice fed 5% EC.

* $P < 0.05$ vs. NC, [†] $P < 0.05$ vs. DC.

Table 3

Effect of EC on glucose-induced insulin secretion in pancreatic islets

EC extract (μ g/mL)	Insulin (μ U/mL)	% baseline
0	52.8 \pm 4.2	100.0 \pm 0.0
25	106.3 \pm 3.6	201.3 \pm 3.1
50	168.9 \pm 8.4	319.9 \pm 6.4
100	124.5 \pm 5.1	235.8 \pm 6.2
300	86.7 \pm 2.9	164.2 \pm 3.1
500	76.8 \pm 2.1	145.5 \pm 2.5

to cholesterol metabolism. We examined the insulinotropic and lipid lowering potential of EC in STZ-diabetic mice. To our knowledge, this is the first time to show the protective effect on pancreatic β -cell and hypolipidemic effect of EC in diabetic mice.

The change in blood glucose levels in response to an oral glucose load has been used clinically for the diagnosis of diabetes and in research to evaluate the hypoglycemic agents. In OGTT study, EC supplementation exhibited significant reduction in blood glucose levels combined with a significant improvement in glucose tolerance. After an oral ingestion of glucose, the maintenance of glucose tolerance depends on insulin secretion, hepatic glucose production, glucose absorption in the digestive tract, and glucose uptake by peripheral tissues^[15]. In the present study, the anti-hyperglycemic activity of EC could be due to its insulinotropic action, as increased insulin secretion in isolated pancreatic islet cells with EC treatment and elevated serum insulin levels in EC treated diabetic mice were observed. Histopathological examination of pancreatic sections also revealed degenerated and necrotic cells in DC mice, while EC treatment significantly alleviated these abnormalities and increased β -cell mass. In support of this association, β -cell death and a reduction in number of islets were observed in pancreas of diabetic rats and hypoglycemic plant extract control the β -cell damage and increased the number of islets^[16,17]. Therefore, the possible mechanism by which EC brings about its hypoglycemic effect may be by increasing the insulin level from the protective effect of pancreatic islets to prevent the loss of β -cell mass, and stimulation of insulin secretion from the remaining β -cells. The increase in serum insulin level after EC treatment could be linked to more than one mechanism. The possible mechanism includes the quantitative changes in pancreatic β -cells by differentiation and proliferation of residual pancreatic β -cells. It may be also attributable to increased insulin synthesis or secretion by residual β -cells similar to that observed after glibenclamide administration. EC also acts as a hepatoprotective agent as shown in liver function marker enzymes, suggesting enhanced transport of blood glucose to peripheral tissues and its utilization, which may be another mechanism of action.

Since lipid abnormalities is the major cause of cardiovascular disease in diabetes, the ideal treatment for diabetes should have a favourable effect on the lipid profile in addition to glycemic control. In views of this, we investigated the effect of EC on serum and hepatic lipid parameter. The altered lipid and lipoprotein profiles were significantly reversed after 4 weeks of EC supplementation to the diabetic mice. Insulin insufficiency is responsible for the derangement of lipid and lipoprotein metabolism^[18]. Insulin decreases TG levels and increases HDL-C through activation of lipoprotein lipase^[19,20]. Insulin also increases receptor-mediated removal of LDL-C and hence decreased activity of insulin during diabetes leads to increased levels of serum LDL-C and consequently hypercholesterolemia^[21]. Therefore, significant control in the serum lipid levels in EC treated diabetic mice might have been due to the insulinotropic action upon EC administration. On the other hand, decreased glucose disposal during diabetes leads to

increased utilization of fatty acids for energy production, which consequently results in increased formation of acetyl coenzyme A and thus of lipids. Hence, the increased insulin levels brought about by EC supplementation indicates the possible effect on sensitizing tissues like skeletal muscle and adipose tissue for uptake of glucose and thus protecting lipid formation.

The increased TG and TC levels and decreased HDL-C are known factors associated with coronary heart disease (CHD)^[22,23]. As EC supplementation produced a favourable effect on these factors, this suggests that it may help to prevent the progression of CHD. Recent studies suggest that TG itself is independently related to CHD, and that most of the anti-hypercholesterolemic drugs do not decrease TG levels^[24,25]. However, EC lowered TG as well as TC and LDL-C to near normal level after 4 weeks of administration. This strong effect on diabetic hyperlipidemia could be through its control on hyperglycemia, because the level of glycemic control is the major determinant of TG and VLDL-C concentrations^[26–32].

An elevated ALT and AST appears to be a consequence of hepatocyte damage, and thus is considered a result of insulin resistance^[33]. Our data showed that EC had a curative effect on liver damage indicated by normalized serum AST and ALT levels, liver lipid levels, and hepatic histological observation in STZ-diabetic mice after EC supplementation. Therefore, improvement of the body weight loss in diabetic mice with EC supplement might be from the beneficial effect on the metabolic state in diabetes via improved pancreatic and liver function.

Natural polyphenols are abundant constituents of seaweeds, and EC has been known as a polyphenol-rich seaweed^[34]. It has been reported that polyphenols from various plants induce pancreatic insulin secretion^[35], and improve dyslipidemia in animal models^[36]. Although the major components that possess the activity in the EC were not precisely identified in this study, it would be of considerable interest to further elucidate the mechanism and component(s) underlying the action of EC.

In conclusion, this study clearly demonstrated that treatment with EC ameliorates hyperglycemia and improves glucose handling capacity in STZ-diabetic mice in a dose-dependent manner, at least in part, by preventing loss of β -cell mass and increase of insulin secretory capacity. This is especially important in preclinical models of type 2 diabetes that are characterized by a progressive decline of pancreatic function due to subsequent β -cell failure and loss of β -cell mass. In addition, EC treatment is a potent and efficacious lipid-lowering agent in diabetic mice.

Conflict of interest statement

We declare that we have no conflict of interest.

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