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Beneficial Effect of Seaweed on High-fat Diet-induced Oxidative Stress and Insulin Resistance in Rats

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Abstract Effect of extracts from *Capsosiphon fulvescens*, *Hizikia fusiforme*, and *Undaria pinnatifida* blades on development of oxidative stress and insulin resistance in rats fed a high-fat diet (HFD) were studied. Sprague-Dawley rats were fed a normal diet, HFD, and HFD-supplemented with *C. fulvescens*, *H. fusiforme*, or *U. pinnatifida* blades for 8 weeks. Supplementation with *C. fulvescens* and *H. fusiforme* extracts significantly (p<0.05) elevated the plasma HDL-cholesterol concentration in HFD-fed rats, compared with controls. The plasma thiobarbituric acid reactive substance level was significantly (p<0.05) lower in *H. fusiforme* and *U. pinnatifida* blade group rats than in HFD group rats. *C. fulvescens*, *H. fusiforme*, and *U. pinnatifida* blade extracts significantly (p<0.05) attenuated the effects of the HFD on plasma total antioxidant and superoxide dismutase activities, and on development of insulin resistance. Consumption of seaweed extracts increased antioxidant effects and improved insulin resistance in HFD-fed rats.

Keywords: seaweed, antioxidant, hyperlipidemia

Introduction

Diabetes mellitus, a metabolic disease resulting from a defect in insulin secretion, insulin action, or both, now affects more than 220 million people worldwide and this number is expected to increase to more than 400 million by 2030 (1). This disease is divided into the 2 major categories of insulin dependent diabetes mellitus (type 1) and non-insulin dependent diabetes mellitus (type 2). The prevalence of diabetes is approximately 10% in Koreans, 90% of whom represent type 2 diabetes (2). Dietary fat is calorically dense, extremely palatable, and causes less satiety than carbohydrates and proteins (3). It is widely accepted that a high level of fat in the diet is detrimental to health. Consumption of a high-fat diet increases the incidence of diabetes, hypertension, and other degenerative disease (4,5). The extent to which diabetes mellitus is induced by diet varies depending on the length of the food intake period, the types and levels of dietary fat, and/or the presence of other modifications in dietary ingredients (4,5).

Recent evidence from high-fat diet induced obesity-associated rat models of oxidative stress and insulin resistance provides support for the utility of interventions that reduce oxidative stress levels and oxidative damage for improvement of insulin action on whole-body and skeletal muscle glucose disposal (6). Antioxidant treatments in animal models clearly mediate improvements in glucose tolerance and insulin sensitivity associated with enhanced functionality of skeletal muscle glucose transport systems (6). Natural antioxidants with fewer side effects have received considerable attention as replacements for synthetic antioxidant components.

Seaweed, an important marine resource, has been used traditionally as a food supplement in Korea and other Asian countries. In recent years, seaweed has been reported to contain antioxidant components and also to exhibit biological activities. For example, extracts from *Ecklonia cava* exerted beneficial antioxidant defense actions against hyperglycemia and improved glucose tolerance and insulin sensitivity (7). More recently, fucoxanthin-rich extracts prepared from *Phaeodactylum tricornutum* have been reported to exert anti-obesity and antioxidant effects in mice fed a HFD (8).

Capsosiphon fulvescens is a filamentous green seaweed traditionally used for centuries in the southwestern regions of Korea as a functional food (9). *Hizikia fusiforme* is a common edible brown seaweed belonging to the *Sargassaceae* family that has anticoagulant, antihypertensive, and immunomodulating effects (10). *Undaria pinnatifida* is also a common edible brown seaweed that is plentiful on the shores of the Korean peninsula. Biological activities of *U. pinnatida* extracts reported to date include antimicrobial, anticoagulant, and anti-obesity actions (11). Although a number of studies have been carried out to investigate the biological activities of seaweed (9-11), systematic studies of beneficial effects on health from the viewpoint of antioxidant activity

in vivo are lacking. Therefore, in this study, the effect of extracts from *C. fulvescens*, *H. fusiforme*, and *U. pinnatifida* blades on insulin resistance and antioxidant status were evaluated in rats fed a high-fat diet.

Materials and Methods

Chemicals 1,1,3,3-Tetraethoxypropane, trichloroacetic acid, Trolox, guanidine hydrochloride, 2,4-dinitrophenylhydrazine, myoglobin from the equine heart, sodium dodecyl sulfate, phosphate-citrate buffer, malondialdehyde, *n*-butanol, and 5,5'-dithiobis 2-nitrobenzoic acid were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals and solvents used were of standard analytical grade.

Seaweed materials *C. fulvescens*, *H. fusiforme*, and *U. pinnatifida* blades were collected from a clean beach near Wando, Korea in July of 2012. Fresh seaweed was washed with tap water and dried in a drying oven (DS80-1; Dasol Scientific, Hwaseong, Korea) at 50°C. Seaweed samples were then ground using a grinder (DA505; Rtlon, Seoul, Korea) and sieved (standard testing sieve, aperture of 300 μm) (Chung Gye Sang Gong Sa, Seoul, Korea) to obtain a fine powder. Powder samples were sealed in zipper bags (25×30 cm) (Cleanwrap, Seoul, Korea) and stored at −20°C (DY-1750RF; Daeyeong, Ansan, Korea) prior to extraction.

Preparation of lyophilized seaweed extracts Powdered seaweed samples were mixed with distilled water (1:25, w/v) and subjected to extraction using an autoclave (JSAC-80; Js Research, Gongju, Korea) at 121°C for 3 h. The filtrate was collected in bottles after vacuum filtration (A-3S; Tokyo Rikikai, Tokyo, Japan). Seaweed filtration residues on filter paper were collected and subjected twice to re-extraction. Filtrates from each extraction were mixed and concentrated at 60°C using a rotary evaporator (N-1000; Tokyo Rikikai) and freeze-dried at −130°C using a freeze dryer (FDCF-12012; Operon, Gimpo, Korea) to eliminate residual water. Lyophilized seaweed extracts were sealed in plastic bags (25×30 cm) (Cleanwrap) and stored at -80°C (Revco Ult 1786-5-D14; Thermo, Asheville, NC, USA). Freeze-dried seaweed extracts were reconstituted in distilled water for oral administration. The total phenolic contents of. *H fusiforme* extracts were highest (39.01 mg of gallic acid equivalents; GAE/g), followed by *U. pinnatifida* blades (30.85 mg of GAE/g) extracts, and *C. fulvescens* (12.44 mg of GAE/g) extracts (12). The major phenolic compounds in *H. fusiforme* extracts were ferulic and *p*-coumaric acids (12). Extracts from *C. fulvescens* contained the phenolic compounds caffeic acid, protocatechuic, chlorogenic, catechin, and epicatechin (12). *U. pinnatifida* blade extracts contained the phenolic compounds syringic and protocatechuic acid (12).

Animals and diets Forty healthy male Sprague-Dawley rats (5 weeks old) were obtained from Joong Ang Lab Animal (Seoul, Korea) and were housed in clean polypropylene cages (2 rats to a cage) placed in a room at 23±2°C and 50±5% relative humidity with a 12 h dark and 12 h light cycle (light on at 6:00 am every day). After a week of acclimatization to laboratory conditions, rats were randomly divided into 5 dietary groups (n=8) with approximately equal mean group body weights. Two groups were fed with either a normal control diet (CON) (10% kcal fat) (38057; Purina Inc., Seongnam, Korea) or a high-fat diet (HFD) (60% kcal fat) (D12492; Research Diets, New Brunswick, NJ, USA). The other 3 groups were fed by gavage with a high-fat diet supplemented with *C. fulvescens* (CF), *H. fusiforme* (HF), or *U. pinnatifida* blades (UPB) at a dosage of 400 mg/kg of body weight daily. CON and HFD groups rats were gavaged with the same volume of water once daily for 8 weeks. The extract dosage was adjusted weekly according to body weight. Rats were allowed free access to food and water during the experimental period of 8 weeks. Food consumption and body weight were measured using an electronic balance daily and weekly, respectively. After 8 weeks of feeding and seaweed extract administration, animals in all groups were starved for 16 h and anatomized under anesthesia using diethyl ether. Blood was collected in tubes containing the anticoagulant lithium heparin to obtain plasma. After exsanguination, organs and adipose tissues were collected, snap-frozen in N_2, and stored at −80°C (Revco Ult 1786-5-D14; Thermo). All institutional and national guidelines for the care and use of laboratory animals were followed. Protocols were reviewed and approved by the Institutional Animal Care and Use Committee of the Mokpo National University (Mokpo, Korea).

Biochemical analysis Plasma concentrations of triglycerides (TG), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C) were measured enzymatically using commercial kits (Asan Pharm Co., Ltd., Seoul, Korea). The low-density lipoprotein cholesterol (LDL-C) concentration was calculated using the Friedewald formula (13): LDL-C=TC−HDL-C−TG×0.2. Hepatic total lipids were quantified following the method of Folch *et al*. (14). The plasma level of glucose was measured using an automatic analyzer (Express Plus; Chiron Diagnostics, East Walpole, MA, USA). The plasma insulin level was assayed using a rat-specific ELISA kit (TMB; Shibayagi Co., Shibukawa, Japan). The reaction was stopped with the addition of an acidic stop solution and the absorbance was read at 450 nm (U-1100; Hitachi, Tokyo, Japan). The homeostasis model assessment of basal insulin resistance (HOMA-IR) was used for calculation of an index from the product of fasting glucose (mg/dL) and insulin levels (μU/mL) divided by 2,430 (15). Lower HOMA-IR values indicated greater insulin sensitivity and higher HOMA-IR values indicated insulin resistance.

Oral glucose tolerance testing (OGTT) One day before termination of experiments, animals were subjected to oral glucose tolerance testing. All rats were fasted for 12 h prior to testing, followed by oral administration of glucose (2 g of glucose/kg of body weight.) Blood samples were collected from the tail vein using a small needle at 0 min before glucose administration and at 30, 60, 90, and 120 min

following glucose administration for determination of blood glucose levels.

Plasma total antioxidant and superoxide dismutase activities The plasma total antioxidant activity (TAC) was measured using a TAC assay kit (CS0790; Sigma) according to the manufacturers' instructions and expressed as Trolox equivalents. The principle of the assay is that, in the presence of hydrogen peroxide, metmyoglobin forms ferryl myoglobin radicals that oxidize 2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) to produce the radical $ABTS^{\cdot+}$ cation. ABTS is colorless but, when $ABTS^{\cdot+}$ is produced, a soluble green colored chromogen is formed that can be quantified spectrophotometrically at 750 nm. Antioxidants suppress production of the radical cation in a concentration dependent manner and the color intensity decreases proportionately. Trolox, a water soluble vitamin E analog, is used in the assay as a standard. The superoxide dismutase (SOD) activity was calculated using a SOD Assay Kit (19160; Sigma) that included Dojindo's highly water-soluble tetrazolium salt, 2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium (WST-1), and monosodium salt. Water-soluble formazan dye is produced upon reduction with the superoxide anion. Twenty μL of diluted plasma was incubated with WST-1 and xanthine oxidase to produce the superoxide anion and the absorbance value at 450 nm was measured (U-1100; Hitachi).

The plasma thiobarbituric acid reactive substance (TBARS) content The plasma TBARS assay is used for indirect measurement of oxidative stress based on calculation of lipid peroxidation species in the body (7,8). Briefly, 100 μL of plasma was placed into tubes to which 0.5 mL of trichloroacetic acid was added. After vortexing (Vortex Genie 2; Scientific Industries, New York, NY, USA) vigorously for 1 min, 0.2 mL of thiobarbituric acid was added to tubes, which were then kept at 95°C (MSH-20A; Daihan Scientific, Wonju, Korea) for 60 min. Tubes were then put in ice-cold water to stop the reaction. *n*-Butanol (800 μL) was then added, followed by vigorous vortexing (Vortex Genie 2; Scientific Industries) as the reaction mixture cooled to room temperature. The optical density of the supernatant (100 μL) obtained via centrifugation (MF-80; Hanil Science, Incheon, Korea) at 6,000×*g* for 10 min was measured at 532 nm (U-1100; Hitachi). Values for malondialdehyde (MDA) products were calculated based on comparison with an MDA standard curve and results were expressed as nmol/mL of plasma.

Determination of the carbonyl content in plasma Amounts of protein carbonyls were measured following the procedure described by Reznick and Packer (16) using dinitrophenylhydrazine (DNPH) reagent and a spectrophotometric method. Ten mM DNPH (4 mL) in 2.5 M HCl was added to 1 mL of a 1/5 diluted plasma sample in a tube. In another tube serving as a blank, 4 mL of 2.5 M HCl was added to 1 mL of an identical sample. Tubes were incubated at 25°C under darkness for 1 h with swirling every 15 min. Protein was precipitated using 5 mL of 20% (w/v) trichloroacetic acid and pellets were washed once with 4 mL of 10% (w/v) trichloroacetic acid and 3 times with 4 mL of an ethanol/ethyl acetate mixture (1:1) to remove free DNPH and lipid contaminants. Washing was performed using mechanical disruption of pellets in the washing solution using a small spatula, and re-pelleting was done using centrifugation (MF-80; Hanil Science) at 6,000×*g* for 5 min. Finally, precipitates were dissolved in 2 mL of a 6 M guanidine-HCl solution (in a 20 mM potassium phosphate buffer, pH 2.3) and the absorbance was measured at 370 nm (U-1100; Hitachi). Results were expressed as nmol/mL of plasma using a molar extinction coefficient of 22,000/M/cm.

Statistical analysis Statistical analyses were performed using GraphPad Prism, version 4.0 for Microsoft Windows (GraphPad Software, San Diego, CA, USA). Data were reported as a mean± standard deviation (SD). Mean values of groups were compared using a one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test, and statistical significance was set at $p<0.05$.

Results and Discussion

Effect of seaweed extracts on body weight, food intake, and the food efficiency ratio Rats fed a HFD exhibited a significantly ($p<0.01$) heavier body weight than CON rats at the end of the experimental period. Initial body weights were identical between the 2 groups (Table 1). The cumulative body weight gain at week 8 in HFD group rats was significantly ($p<0.05$) higher than for CON group rats (Table

Table 1. Effects of seaweed extract supplementation on body weight gain, food intake, and the food efficiency ratio of rats fed a HFD

	CON[1)]	HFD	CF	HF	UPB
Initial weight (g)	163.38±2.89	164.23±2.33	164.10±2.97	163.80±4.21	162.85±1.92
Final weight (g)	446.75±5.82$^{\#\#}$[2)]	516.25±8.77**	463.00±8.54$^{\#\#}$	496.00±7.72*	490.25±10.40*
Weight gain (g/day)	5.23±0.16$^{\#}$	6.13±0.27*	5.33±0.20$^{\#}$	5.90±0.10	5.93±0.14
Food intake (kcal/day)	74.28±0.53$^{\#}$	88.87±1.84*	83.84±0.15*	83.94±2.10*	84.21±0.42*
FER	0.21±0.01$^{\#\#\#}$	0.33±0.05***	0.32±0.01***	0.35±0.01***	0.35±0.02***

[1)]CON: group fed a control diet; HFD: group fed a high-fat diet; CF: group fed a HFD with added *Capsosiphon fulvescens*; HF: group fed a HFD with added *Hizikia fusiforme*; UPB: group fed a HFD with added *Undaria pinnatifidia* blades; FER: the food efficiency ratio

[2)]Data represent mean values±SD, *$p<0.05$, **$p<0.01$, ***$p<0.001$ relative to CON, $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ relative to HFD-fed rats, ANOVA followed by Dunnett's Multiple Comparison test.

1). On the other hand, supplementation with seaweed extracts suppressed the body weight gain induced by HFD. After 8 weeks of experimental feeding, the final body weight gain was significantly (p<0.01) lower in CF group rats than that in HFD group (Table 1). This was not due to lower food consumption since the food intake was similar for both HFD and seaweed-fed rats during the 8 week feeding period (Table 1). Food intake values for HFD group rats were significantly (p<0.001) lower than for CON group rats. Therefore, food efficiency ratios for HFD group rats were significantly (p<0.001) higher than for CON group rats (Table 1).

Effect of seaweed extracts on weights of internal organs and adipose tissues Comparisons of liver, kidney, spleen, pancreas, and adipose tissue weights of experimental animals are shown in Table 2. There was no difference in color or appearance of the liver among experimental group rats. The relative weight of the liver in HFD group rats was significantly (p<0.05) higher than for CON group rats (Table 2). Supplementation with seaweed extracts contributed to decreased relative liver weights, compared with HFD group rats. This liver weight decrease probably indicated a beneficial effect of seaweed on liver health that could be related to lipid metabolism (Table 2). Weights of the kidney, spleen, and pancreas did not significantly (p>0.05) differ between control and experimental diet-fed group rats. Retroperitoneal and epididymal fat-pad weights in HFD group rats were significantly (p<0.01) (93 and 59%, respectively) greater than for CON group rats (Table 2). Moreover, retroperitoneal and epididymal fat-pad weights were significantly (p<0.01) decreased in CF group rats (31 and 32%, respectively) compared with HFD group rats (Table 2).

Effect of seaweed extracts on lipid profiles HFD-fed rats showed significantly higher levels of plasma TC (p<0.001) and lower levels of plasma HDL-cholesterol (p<0.01) than those in CON-fed rats (Table 3). Supplementation with seaweed extracts significantly (p<0.05) attenuated HFD-induced elevation in plasma TC concentrations, compared with control rats. Plasma TC levels were decreased in CF, HF, and UPB group rats (approximately 18, 23, and 27%, respectively) compared with HFD group rats (Table 3). The HFD-induced decrease in HDL-cholesterol plasma levels was also significantly (p<0.05) reversed by supplementation of the HFD with seaweed extracts, compared with control rats (Table 3). TG levels in rats from the HFD group were significantly (p<0.05) higher than those in CON group rats (Table 3). Hepatic total lipid levels were significantly (p<0.05) decreased in CF, HF, and UPB group rats (approximately 41, 36, and 38%, respectively) compared with HFD group rats. Supplementation with seaweed extracts had effects for improvement of lipid homeostasis in experimental rats.

Abnormal blood lipid profiles are associated with an elevated risk of coronary heart disease, particularly in individuals with metabolic syndrome or type 2 diabetes. HDL-cholesterol, an essential component

Table 2. Effect of seaweed extract supplementation on weights of internal organs and adipose tissues (unit: g/100 g of body weight)

	Liver	Kidney	Spleen	Pancreas	Epididymal fat	Retroperitoneal fat
CON[1)]	2.96±0.11$^{\#2)}$	0.66±0.02	0.21±0.02	0.24±0.03	1.48±0.07$^{\#\#}$	1.87±0.07$^{\#\#}$
HFD	3.38±0.11*	0.62±0.02	0.17±0.01	0.21±0.01	2.35±0.15**	3.26±0.15**
CF	2.80±0.09$^{\#\#}$	0.63±0.03	0.18±0.01	0.20±0.01	1.60±0.18$^{\#\#}$	2.27±0.18$^{\#\#}$
HF	2.87±0.07$^{\#\#}$	0.61±0.02	0.19±0.01	0.20±0.01	2.01±0.25**	2.84±0.25*
UPB	2.89±0.07$^{\#\#}$	0.61±0.02	0.19±0.01	0.18±0.04	2.16±0.28**	3.01±0.28**

[1)]CON: group fed a control diet; HFD: group fed a high-fat diet; CF: group fed a HFD with added *Capsosiphon fulvescens*; HF: group fed a HFD with added *Hizikia fusiforme*; UPB: group fed a HFD with added *Undaria pinnatifidia* blade

[2)]Data represent the mean values±SD, *p<0.05, **p<0.01 relative to CON, $^{\#}p$<0.05, $^{\#\#}p$<0.01, $^{\#\#\#}p$<0.001 relative to HFD, ANOVA followed by Dunnett's Multiple Comparison test.

Table 3. Effect of seaweed extract supplementation on glucose and insulin concentrations, and lipid profiles in rats

	CON[1)]	HFD	CF	HF	UPB
TC (mg/dL)[2)]	58.65±3.86$^{\#\#\#3)}$	93.37±7.94***	76.95±3.10$^{\#}$	71.93±3.36$^{\#}$	67.80±5.34$^{\#\#}$
HDL-C (mg/dL)	45.36±1.01$^{\#\#}$	35.22±1.17**	41.81±2.52$^{\#}$	43.01±2.68$^{\#\#}$	37.57±1.99*
LDL-C (mg/dL)	8.55±0.56$^{\#\#}$	52.72±2.54**	30.34±1.42$^{\#}$	23.92±1.35$^{\#\#}$	25.33±2.09$^{\#}$
TG (mg/dL)	23.55±1.91$^{\#}$	27.16±2.68*	24.07±3.00	25.10±0.91	24.58±2.19
Glucose (mg/dL)	85.83±3.32$^{\#\#}$	109.17±2.57*	102.50±2.53*	108.33±3.96*	108.67±3.64*
Insulin (ng/mL)	1.51±0.49$^{\#\#\#}$	3.06±0.37***	2.67±0.18**	2.33±0.11$^{\#}$	2.56±0.11**
HOMA-IR	1.59±0.13$^{\#\#\#}$	3.42±0.45***	2.77±0.25$^{\#}$	2.22±0.18$^{\#\#}$	2.73±0.17$^{\#}$
Total lipid (mg/g liver)	29.45±2.67$^{\#}$	48.16±2.34*	28.46±1.76$^{\#}$	30.65±2.58$^{\#}$	29.65±2.09$^{\#}$

[1)]CON: group fed a control diet; HFD: group fed a high-fat diet; CF: group fed a HFD with added *Capsosiphon fulvescens*; HF: group fed a HFD with added *Hizikia fusiforme*; UPB: group fed a HFD with added *Undaria pinnatifidia* blade

[2)]TC: total cholesterol; HDL-C: high-density lipoprotein-cholesterol; LDL-C: low-density lipoprotein-cholesterol; TG: triglyceride; HOMA–IR: homeostasis model assessment for insulin resistance

[3)]Data represent the mean±SD, *p<0.05, **p<0.01, ***p<0.001 relative to CON, $^{\#}p$<0.05, $^{\#\#}p$<0.01, $^{\#\#\#}p$<0.001 relative to HFD, ANOVA followed by Dunnett's Multiple Comparison test.

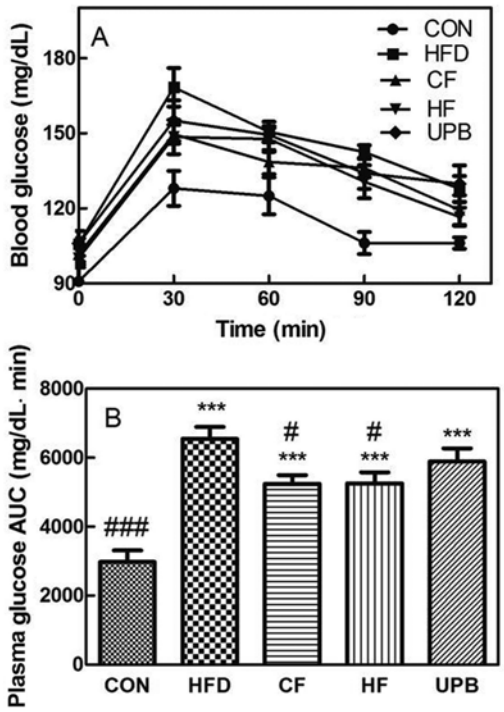

Fig. 1. Effect of seaweed extract supplementation on glucose use in rats fed a HFD.

in reverse cholesterol transport, facilitates translocation of cholesterol from peripheral tissues, such as arterial walls, to the liver for catabolism (17). A number of studies have shown that a diet with a high fat content caused a considerable decrease in HDL-cholesterol levels and an increase in cholesterol levels in rats (4,18).

A decrease in circulating HDL-cholesterol levels and an increase in cholesterol levels in HFD group rats was observed in this study. Seaweed extract supplementation of the HFD for rats showed restoration of lipid parameters back to control levels, indicating that seaweed extracts inhibited absorption of cholesterol, based on a decrease in micellar cholesterol solubility (19).

Effect of seaweed extracts on glucose use and insulin sensitivity Seaweed extract influences on insulin sensitivity were investigated. OGTT was performed after 8 weeks of diet supplementation to determine the effect of seaweed extracts on glucose tolerance in HFD-fed rats (Fig. 1A). Following HFD feeding, animals developed overt glucose intolerance. The integrated plasma glucose concentration, calculated from the area under the curve (AUC), was significantly (p<0.05) elevated in HFD-fed rats, compared with controls (Fig. 1B). Treatment with *C. fulvescens*, *H. fusiforme*, and *U. pinnatifida* blade extracts effectively improved glucose tolerability, reducing AUC values by 20, 20, and 10% in CF, HF, and UPB group rats, respectively, compared to HFD group rats (Fig. 1B). Furthermore, fasting plasma glucose and insulin levels measured at the end of the feeding period revealed that CF, HF, and UPB group rats exhibited reductions in plasma glucose and insulin levels, compared with HFD group rats (Table 3). Along with plasma glucose and insulin levels, HOMA-IR values indicated that insulin sensitivity was significantly (p<0.05) improved in CF, HF, and UPB group rats, compared with control rats (Table 3).

Moderate but significant (p<0.05) elevations in blood glucose levels associated with hyperinsulinemia in rats fed a high-fat diet, compared with controls, indicated insulin resistance. The presence of insulin resistance was further indicated by high HOMA-IR values. HOMA-IR was used because it is a valuable method that shows a strong relationship with a euglycemic-hyperinsulinemic clamp (20) and, therefore, has been proposed for use as an alternative method. High HOMA-IR scores denoted low insulin sensitivity (insulin resistance). Consistent with results reported herein, Chen *et al.* (21) found that the HOMA-IR index was significantly lower in magnesium lithospermate B-treated rats (approximately 20% decrease) than in respective controls (HFD rats), which is indicative of high insulin sensitivity.

Hyperglycemia was exacerbated by the oral glucose load. A significant (p<0.05) increase in the AUC of the glucose concentration after glucose loading during OGTT was seen in HFD group rats, compared with controls, indicating that the ability of insulin to stimulate glucose disposal in peripheral tissues was impaired by a high-fat diet. Plasma glucose and insulin levels, and insulin resistance were favorably modified by seaweed extracts, indicating that seaweed extracts improved high-fat diet induced insulin resistance in part via attenuation of insulin-stimulated glucose disposal impairment in rats. In addition, a previous *in vitro* study demonstrated an α-glucosidase inhibitory activity of *C. fulvescens*, *H. fusiforme*, and *U. pinnatifida* blade extracts (12). A recent study has demonstrated that a medicinal plant with *in vitro* α-glucosidase and α-amylase inhibitory activities reduced peak blood glucose levels and areas under the curve (AUC) in diabetic rats when challenged with oral administration of starch and sucrose (22). Hence, α-glucosidase inhibition may be a mechanism for exertion of antidiabetic activities by seaweed extracts.

Effect of seaweed extracts on oxidative markers The plasma TAC level was significantly (p<0.05) lower, compared with control rats, whereas plasma TBARS levels (p<0.001) and protein carbonyl levels (p<0.01) were significantly (p<0.001) higher in HFD group rats than in CON group rats (Figs. 2-4). The high-fat diet also changed the activities of SOD (Fig. 5). A significantly increased SOD activity was observed in CF (p<0.01), HF (<0.01), and UPB (<0.01) group rats, compared with HFD group rats (Fig. 5). *H. fusiforme* (p<0.001) and *U. pinnatifida* blade extracts (p<0.05) significantly decreased TBARS levels in rats fed a HFD, compared with controls. Analysis of the TBARS concentration is widely used for assessment of lipid peroxidation and oxidative stress in laboratory animals. High dietary fat intake is known to promote formation of free radicals and reactive oxygen species, resulting in lipid peroxidation and oxidative stress, which is associated with development of hyperglycemia (23).

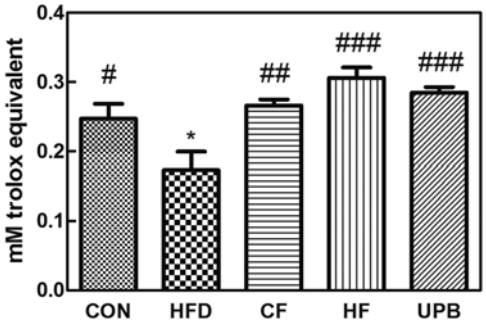

Fig. 2. Effect of seaweed extract supplementation on plasma total antioxidant activity (TAC) in rats fed a HFD.

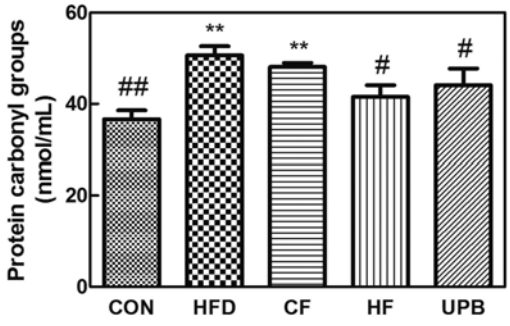

Fig. 4. Effect of seaweed extract supplementation on plasma protein carbonyl levels in rats fed a HFD.

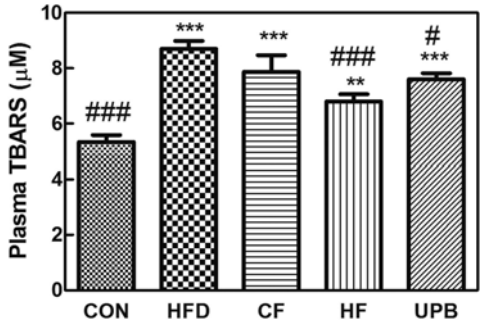

Fig. 3. Effect of seaweed extract supplementation on plasma thiobarbituric acid reactive substance (TBARS) levels in rats fed a HFD.

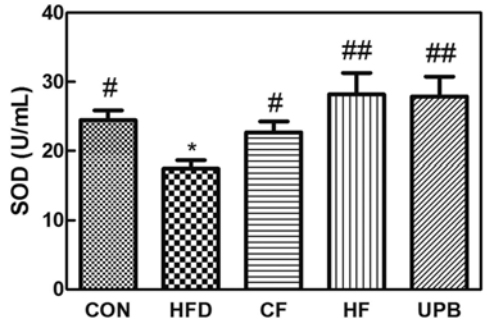

Fig. 5. Effect of seaweed extract supplementation on the superoxide dismutase (SOD) activity in rats fed a HFD.

Supplementation with seaweed extracts also reduced the protein carbonyl level. In particular, the protein carbonyl level in HF group rats was significantly (p<0.05) decreased, compared with HFD group rats (Fig. 4). Decreased levels of plasma TBARS and protein carbonyl and increased activities of SOD and TAC in CF, HF, and UPB group rats indicated an improvement in the *in vivo* anti-oxidative defense status of rats, resulting in less susceptibility to oxidative stress under high-fat diet conditions.

It has been reported that chronic consumption of a high-fat diet contributes to excessive ROS production. An increase in cellular ROS accumulation directly triggers activation of serine/threonine kinase cascades, such as the c-Jun N-terminal kinase and nuclear factor-kappa B that, in turn, phosphorylate multiple targets, including insulin receptor and insulin receptor substrate (IRS) proteins (24). Increased serine phosphorylation of IRS directly decreases its ability to undergo tyrosine phosphorylation and accelerates degradation of IRS-1, causing impaired glucose uptake in muscle, liver, and adipose tissues (24). Therefore, the scavenging effects of free radicals *in vitro* and the antioxidative status *in vivo* are both important. On the other hand, blood is an important substance affected by oxidative stress and assays of blood biochemical markers are of great clinical importance for diagnosis of malfunctioning body organs. Correlative evidence in humans indicates an association between plasma markers of oxidative stress and tissue damage and the degree of insulin resistance (25). Thus, in this study, TAC, SOD, TBARS, and protein carbonyl levels in plasma were used for determination of the antioxidant effects of seaweed extracts *in vivo*.

There is an ongoing balance between antioxidants and ROS for prevention of oxidative stress. When there is an imbalance, ROS can accumulate and trigger oxidative injury from lipid peroxidation and protein oxidation, accompanied by increased toxic product synthesis and cell death. Antioxidant enzymes are capable of scavenging reactive oxygen species and products of lipid peroxidation, thereby protecting cells and tissues from oxidative damage. SOD catalyzes dismutation of radical anions to H_2O_2 and O_2. Numerous studies have shown the importance of SOD in protecting cells against oxidative stress (26,27). In agreement with previous reports (26,27), a significant (p<0.05) decrease in the SOD activity during high-fat diet ingestion was observed, compared with controls. Such a decrease could have been due to feedback inhibition or to oxidative inactivation of enzyme proteins due to excess ROS generation.

Seaweed phenolics constitute a major group of compounds acting as primary antioxidants and free radical terminators (28). In this study, water-based seaweed extracts contained high amounts of polyphenolic compounds and exhibited free radical scavenging activities comparable with values in common food products and in other plant sources (29,30). Caffeic acid was the most abundant phenolic compound found in *C. fulvescens* extracts. In addition, protocatechuic, chlorogenic, catechin, and epicatechin were also found in *C. fulvescens* extracts. The major phenolic compounds in *H. fusiforme* extracts were *p*-coumaric and ferulic acids. *U. pinnatifida* blade extracts contained the phenolic compounds syringic and

protocatechuic acids. Caffeic acid significantly increased superoxide dismutase, catalase, and glutathione peroxidase activities and respective mRNA levels, while lowering hydrogen peroxide and thiobarbituric acid reactive substances levels in erythrocytes and livers of *db/db* mice (31). *p*-Coumaric acid was reported to have a substantial activity for scavenging ·OH and for inhibition of lipid peroxidation, and for reduction of plasma total cholesterol levels *in vivo* (32). Syringic acid displayed a strong antioxidant activity in a dose-dependent manner, with a 50% inhibitory concentration of 0.7 μg/mL (3.54 μM) in an HL-60 cell-based assay that directly examines the ability of test materials to penetrate living cells and inhibit ROS catalysed oxidation of 2,7-dichlorofluorescin (DCFH) to 2,7-dichloro-fluorescein (DCF) (33). It is possible that active phenolic compounds in seaweed extracts improved insulin sensitivity and increased plasma TAC and antioxidant enzyme activities due to antioxidant properties.

In conclusion, *C. fulvescens*, *H. fusiforme*, and *U. pinnatifida* blade extracts elevated plasma total antioxidant and SOD activities and improved blood lipid homeostasis and insulin resistance in HFD-fed rats. *H. fusiforme* and *U. pinnatifida* blade extracts also reversed HFD-induced elevation of lipid peroxide and protein carbonyl levels. Further studies regarding use of seaweed extracts as natural antioxidants, nutraceuticals, and pharmaceutical ingredients are needed.

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