

Full Length Research Paper

Effects of *Salicornia herbacea* L. supplementation on lipid peroxidation and antioxidative protein expression in rat skeletal muscle

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Effects of *Salicornia herbacea* L. supplementation on lipid peroxidation and antioxidative protein expression in the skeletal muscle of rats was investigated in this study. Sixteen 6-week-old Sprague Dawley male rats were randomly divided into a control diet group (CG, $n = 8$) and an experimental diet group (EG, $n = 8$). The rats were given a high-fat diet (CG) or the 5% *S. herbacea* L. supplemented high-fat diet (EG) for 8 weeks. The results showed that the perirenal fat weight in the EG group was slightly lower than in the CG group, but the difference between the groups was not significant. Among the blood components analyzed, triglyceride (TG) level of the EG group was significantly lower than that of the CG group ($p < 0.01$). Malondialdehyde (MDA) level in the EG group was significantly lower than in the CG group ($p < 0.01$). Additionally, the levels of Cu, Zn-superoxide dismutase (SOD), Mn-SOD and glutathione peroxidase (GPx) protein expression in the soleus muscle of the EG group was increased significantly versus the CG group ($p < 0.01$). These results suggest that a diet supplemented with *S. herbacea* L. improved the antioxidative properties of the skeletal muscle of rats.

Key words: *Salicornia herbacea* L., high-fat diet, malondialdehyde (MDA), superoxide dismutase (SOD), glutathione peroxidase (GPx).

INTRODUCTION

Salicornia herbacea L., a halophyte, is a member of the Chenopodiaceae subfamily that is found mostly with *Suaeda japonica* and *Suaeda maritima* on the western and southern coasts of South Korea (Cha et al., 2003; Kim and Song, 1983). *S. herbacea* L., which grows mostly on mudflats with high salinity, stores a larger amount of absorbed salt than terrestrial plants to withstand

osmotic pressure (Netty et al., 2004). This plant is replete in natural minerals, such as K, Ca and Mg, and consists of ~50% essential fatty acids, such as linolenic acid. Of the total amino acids in *S. herbacea* L., ~40% are essential amino acids, and the plant is known to have high value as a healthy functional food source (Min et al., 2002; Shin et al., 2002). Various studies concerning the

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improvement of physiological functions following *S. herbacea* L., treatment have suggested that it has anti-diabetic (Park et al., 2006), anti-obesity (Lee et al., 2012), and anti-cancer (Kong et al., 2008) activities, ameliorated hepatic necrosis (Kim and Kim, 1996), and prevented cardiovascular disease (Lee et al., 2004). Ha et al. (2006) reported that treatment with *S. herbacea* L. reduced the liver malondialdehyde (MDA) content and increased its superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities in rats that were ovariectomized to induce oxidative stress. Furthermore, it has been suggested that *S. herbacea* L. is a natural antioxidant because its dicaffeoylquinic acid derivative and flavonoid glucosides show high levels of antioxidant activity (Kim et al., 2011). Chronic intake of a high-fat diet causes obesity by increasing the body and fat-tissue weights, causes diabetes by increasing glucose levels, and increases cardiovascular disease risk factors by causing hypertension, which causes kidney dysfunction; these metabolic disorders are also closely associated with increasing levels of free radicals in various organs (Vincent et al., 2001; Raffaella et al., 2008; Amin et al., 2011). It is known that free radicals reduce antioxidant activities, causing signs of aging, various diseases, cell damage through intracellular deoxyribonucleic acid (DNA) damage and mutations, and the production of peroxides (Niess et al., 1999). Lipid peroxidation leads to the formation of a number of by products, such as MDA, which can cause the cross-linking and polymerization of membrane components and thus alter intrinsic membrane properties, such as iron transport and enzymatic activity and can cause mutagenic lesions by reacting with the nitrogenous bases of DNA (Fridorich, 1986).

However, to protect against the potentially damaging effects of free radicals and to effectively diminish the free radical content, the human body possesses several antioxidant enzymes, such as SOD, GPx, and CAT. SOD can convert superoxide radicals into hydrogen peroxidase, and the hydrogen peroxidase that is produced is eliminated by CAT and GPx, which are responsible for converting hydrogen peroxidase to water (Kruz et al., 2004). Furthermore, it is well established that non-enzymatic antioxidants, such as vitamin-C (ascorbic acid), vitamin-E (α -tocopherol), GSH (glutathione), flavonoids, and β -carotene, play important roles in diminishing the free radical content in the human body (Valko et al., 2007).

The human body continuously generates free radicals during metabolism in the resting condition (Kojda and Hambrecht, 2005), and oxygen uptake and utilization during strenuous exercise may be increased by 10 to 15 times versus the resting state, leading to the development of free radicals and lipid peroxidation in muscle tissues, resulting in oxidative stress (Clarkson, 1995). However, the antioxidative functions of muscle tissue may be reduced by a long-term high-fat diet. Sreekumar et al.

(2002) reported that supplementation of a high-fat diet reduced the expression of Cu, Zn-SOD and Mn-SOD mRNAs in the muscle tissue of rats, leading to a reduction in antioxidant enzymatic activity. Bonnard et al. (2008) reported that 16 weeks of high-fat diet supplementation increased the serum H_2O_2 level and reduced the number of intra-myofibrillar mitochondria compared with control-diet group. Recently, various research studies on enhancing antioxidant functions using natural substances have been performed (Kwon et al., 2012). Although, *S. herbacea* L. has been reported to be a natural antioxidant source in many studies, its antioxidant effects on the oxidative stress in muscle tissues caused by obesity have not been clearly demonstrated. Thus, this study was conducted to determine the effects of 8 weeks of *S. herbacea* L. dietary supplementation on lipid peroxidation and antioxidative protein expression in the skeletal muscles of rats.

MATERIALS AND METHODS

Experimental animals

All of the experimental protocols were approved by the Animal Study Committee of Sunmoon University. After an acclimatization period of 1 week, sixteen 6-week-old male Sprague Dawley rats (Samtaco Bio Korea, Hwaseong, Korea) were divided randomly into two groups: a control diet group (CG: high-fat diet group, $n = 8$) and an experimental diet group (EG: 5% *S. herbacea* L.-supplemented diet group, $n = 8$). All animals were given free access to tap water and food for 8 weeks, and housed in groups of two per cage under controlled temperature ($23\pm 1^\circ\text{C}$) and relative humidity ($50\pm 5\%$) conditions. The light/dark cycle was controlled automatically (alternating 12-h periods), and lighting was begun at 8:00 pm.

The amount of food intake was measured daily and body weight was measured weekly. Food efficiency ratio (FER) was calculated as the total weight gained divided by the total food intake for the experimental period. At the end of the experimental period, the rats were sacrificed by exsanguination, drawing blood from the left ventricle under light diethyl ether anesthesia after fasting for 12 h. Blood samples were taken from the left ventricle, and the serum was obtained by centrifuging the blood ($700\times g$, 20 min, 4°C). The soleus muscles were dissected and immediately snap-frozen in liquid nitrogen. The serum samples and the soleus muscles were stored at -70°C until they were analyzed.

Preparation of experimental diet

As shown in Table 1, the high-fat diet consisted of 20% protein, 48% carbohydrate and 20% fat, modified from a previous study (Kim and Park, 2008) and was based on AIN-76G. The 5% *S. herbacea* L. supplemented diet was prepared by substituting a portion of the fiber component of the high-fat diet.

Serum components and muscle MDA analysis

The serum glucose, triglyceride (TG), total cholesterol (TC), and high density lipoprotein cholesterol (HDL) levels were analyzed using enzymatic kits (Asan Pharmaceutical Co, Yongin, S. Korea).

Table 1. Composition of the experimental diet (g/kg diet).

Ingredient	High-fat diet	Diet with 5% <i>Salicornia herbacea</i> L. added
Casein	200	200
Starch	111	111
Sucrose	370	370
Lard	170	170
Corn oil	30	30
Cellulose	50	.
Vitamin mix.	12	12
Mineral mix.	42	42
Cholesterol	10	10
D,L-methionine	3	3
Choline barbiturate	2	2
Tert-butylhydroquinone	0.04	0.04
<i>Salicornia herbacea</i> L.	.	50

MDA levels in the soleus muscle were determined calorimetrically using thiobarbituric acid according to the method of Buege and Aust (1978).

Western blot analysis

For protein expression analysis, soleus muscles were homogenized on ice using a Polytron homogenizer in 19 volumes of 20 mmol/L Tris-HCl buffer (pH 7.5) containing 5 mmol/L ethylenediaminetetraacetic acid (EDTA), 2 mmol/L phenylmethylsulfonyl fluoride (PMSF), and a protease inhibitor cocktail (Sigma, St Louis, MO, USA). The homogenates were centrifuged (1,200×g, 10 min) and the supernatant was collected and then recentrifuged (10,000×g, 10 min). The supernatants were used to determine the Cu, Zn-SOD and GPx proteins expression levels.

The pellets were resuspended in homogenizing buffer and used to determine the Mn-SOD protein expression level. The protein concentrations were determined using the Bradford method (Bio-Rad, Hercules, CA, USA) with bovine serum albumin as the standard. All of the protein extraction procedures were conducted at 4°C. An aliquot of tissue extract containing 20 µg of protein was mixed with an equal volume of Laemmli buffer, heated at 100°C in a heating block for 5 min, and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis along with a mixture of molecular-weight standards (Bio-Rad, Hercules, CA, USA). After electrophoresis, the proteins were transferred to a PVDF membrane (Millipore, Bedford, MA, USA) in a semi-dry blotting apparatus (Bio-Rad). After treating with blocking buffer (PBS containing 10% skim milk) for 90 min, the membrane was incubated with primary polyclonal antibodies for 2 h (SOD1, SOD2, GPx, Santa Cruz Biotechnology, Santa Cruz, CA, USA), followed by five 10-min washes with PBS (5% Tween 20).

The membrane was then incubated with HRP-conjugated anti-goat IgG or anti-rabbit IgG (Santa Cruz Biotechnology) for 1 h, followed by five 10-min washes with PBS (5% Tween 20). The target proteins were detected using an ECL kit (Amersham Pharmacia Biotech, Piscataway, NJ, USA). The films were photographed and the protein bands of interest were quantified with band analyzer software (Bio-Rad).

Statistical analysis

All data were analyzed using the Statistical Package for Social Sciences (SPSS) software (ver. 16.0 for Windows). The data are expressed as means ± standard error (SE), and values were analyzed with the independent-samples *t*-test. Statistical significance was set at $p < 0.05$.

RESULTS

Changes in the body weight, food efficiency ratio (FER), and fat weight

As shown in Table 2, the initial and final body weights of both groups were 250 and ~400 g, respectively. Thus, no weight reduction was evident with *S. herbacea* L. supplementation. In the fat weight, perirenal fat weight (PFW) in EG (6.5±0.57 g) was decreased slightly versus the value in CG (6.9±0.57 g), but the difference was not significant. The epididymal fat weight (EFT) in EG (6.3±0.47 g) also showed no significant difference versus the value in CG (6.3±0.38 g). The FER also showed no significant difference between EG (0.19±0.007) and CG (0.17±0.005).

Changes in serum components

As shown in Table 2, there was no significant difference in the serum glucose, TC, and HDLC levels between the groups. However, the TG level of the EG group was reduced by 30% and showed a significant difference compared with the CG ($p < 0.01$).

Changes in muscle MDA content

As shown in Figure 1, the MDA content of EG group was

Table 2. Changes in the weight, FER and serum components.

Parameter	CG	EG
Initial body weight (g)	251.1 ± 2.51	250.4 ± 2.25
Final body weight (g)	398.4 ± 9.90	399.6 ± 13.10
Food efficiency ratio	0.17 ± 0.005	0.19 ± 0.007
Perirenal fat weight (g)	6.9 ± 0.57	6.5 ± 0.57
Epididymal fat weight (g)	6.3 ± 0.38	6.3 ± 0.47
Glucose (mg/dl)	130.3 ± 1.66	131.7 ± 1.80
Triglyceride (mg/dl)	26.1 ± 1.39	18.4 ± 1.32**
HDLC (mg/dl)	63.8 ± 1.65	58.1 ± 3.98
TC (mg/dl)	22.8 ± 1.20	23.2 ± 0.86

The data are expressed as the mean ± SE; CG: Control diet group; EG: Experimental diet group; HDLC: high-density lipoprotein cholesterol; TC: total cholesterol; **p < 0.01 compared with the CG.

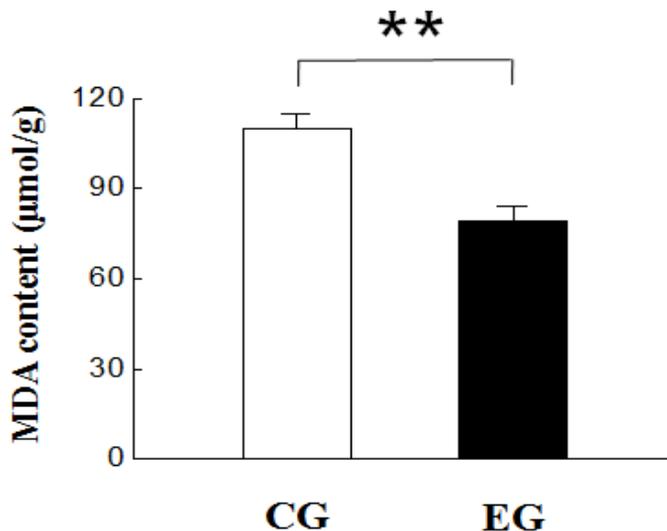


Figure 1. Changes in the MDA content of the soleus muscle. CG: Control diet group; EG: Experimental diet group; **p<0.01.

reduced by 28% and showed a significant difference versus the CG after 8 weeks of supplementation with *S. herbacea* L. (p < 0.01).

Changes in muscle Cu, Zn-SOD, Mn-SOD, and GPx protein expression

The Cu, Zn-SOD (A), Mn-SOD (B), and GPx (C) protein expression levels in soleus muscle are as shown in Figure 2. The Cu, Zn-SOD, Mn-SOD, and GPx protein expression levels in the EG increased approximately to 44, 16, and 33%, respectively, compared with CG, and all of these proteins showed significant differences (p < 0.01).

DISCUSSION

The aim of this study was to determine the effects of 8 weeks of *S. herbacea* L. dietary supplementation on the levels of lipid peroxidation and antioxidative protein expression in the skeletal muscles of rats that were fed a high-fat diet. The results showed that, although the body weights and the values for FER, PFW, and EFW of the groups were not significantly different at the end of the treatment period, the PFT value tended to be lower in the EG versus the CG. Previous studies have reported that long-term *S. herbacea* L. supplementation suppressed differentiation of adipocytes and body weight gain *in vitro* and *in vivo*, respectively. Kong and Seo (2012) reported that 3T3-L1 adipocytes with isohamnetin 3-O-β-D-glucopyranoside obtained from *S. herbacea* L. for 6 days suppressed adipocytic differentiation through reduced expression of peroxisome proliferator-activated receptor-γ (PPAR-γ), CCAAT/enhancer-binding proteins (C/EBPα), and sterol regulatory element-binding protein 1 (SREBP1). Furthermore, it has been reported that 4 weeks of experimental drink supplementation, implemented by adding a solution of an extract of *S. herbacea* L. (0, 5, 10, 15, and 20%) to water, inhibited weight gain compared with a control group and that providing a higher concentration of the extract solution had a greater effect in an animal study (Jo et al., 2002).

However, another study reported that 10 and 20% of *S. herbacea* L. dietary supplementation had no effect on reducing body weight gain, similar to the present results (Kim, 2007). It is well known that gaining fat tissue is a more significant risk factor for obesity than weight gain per se (Park et al., 2010). In particular, the accumulation of visceral fat, which is the most important risk factors in abdominal obesity, is correlated with life style-related diseases, such as diabetes, hyperlipidemia, and coronary heart disease (Despres and Lemieux, 2006). In the present study, although the PFW values were not significantly

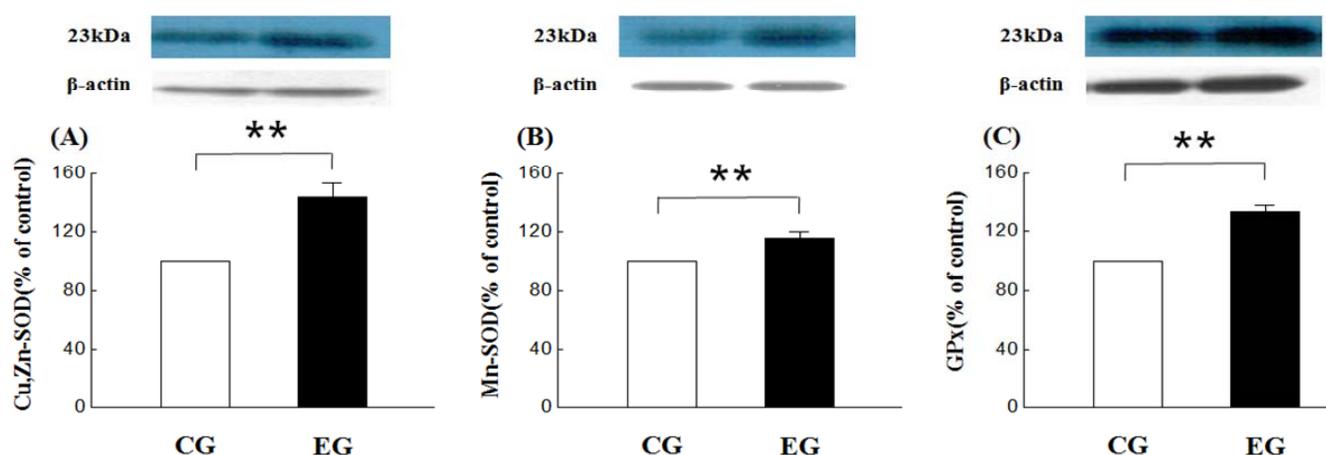


Figure 2. Changes in the Cu,Zn-SOD, Mn-SOD and GPx protein expression levels in the soleus muscle. (A) Mn-SOD, (B) Cu,Zn-SOD, (C) GPx. CG: Control diet group; EG: Experimental diet group; ** $p < 0.01$.

significantly different, the EG value of PFW tended to be lower than CG. This result suggested that dietary supplementation of *S. herbacea* L. had a positive effect in reducing the accumulation of PFW. However, further studies are needed to fully determine the effects of *S. herbacea* L. supplementation on obesity under a different experimental conditions, including using different doses and periods of supplementation.

The serum component results showed that only the TG level in the EG was significantly lower than that of the CG, with no significant difference in glucose, TC, and HDLC levels. Similar to the present study, Kim (2007) found that 4 weeks of a *S. herbacea* L. supplemented diet had no effect on serum TC or HDLC levels in rats. Additionally, 350 mg/kg of *S. herbacea* L. supplementation for 10 weeks showed no changes in serum glucose levels of rats fed a high-fat diet, whereas the serum TG level was significantly reduced versus that of the non-supplemented group (Park et al., 2006). Seo et al. (2012) also reported that although 4 weeks of *S. herbacea* L. supplementation had no effect on serum TC levels, the serum TG level was reduced significantly. It has also been reported that treating 5% STZ (streptozotocin)-induced diabetic rats for 5 weeks with a *S. herbacea* L.-supplemented diet significantly reduced their serum TG level compared with a normal diet group (Bang et al., 2002), supporting the present findings.

The hypolipidemic effect of *S. herbacea* L. is due to abundant amount of fiber which decreases the serum TG level by promoting the disintegration of chylomicrons and VLDL, a major carrier of TG, by activating lipoprotein lipase in capillary walls (Vigne et al., 1987). Thus, the hypolipidemic effect of *S. herbacea* L. is due to stimulating fat excretion and delaying fat absorption, as a result of the abundant soluble fiber content (Han and Kim, 2003).

Regarding oxidative stress, lipid peroxidation involves the oxidation of the unsaturated fatty acids in the cell membranes by free radicals, and MDA is widely used as a lipid-peroxidation indicator (Bang et al., 2002). High-fat diet-induced obesity leads to oxidative damage due to an increase in reactive oxygen species (ROS) levels (Kuratko and Pence, 1991). ROS can react with polyunsaturated fatty acids (PUFAs), resulting in cell membrane dysfunction by increased lipid peroxidation and oxidative damage (Mahboob et al., 2005). Noeman et al. (2011) reported that 16 weeks of high-fat dietary supplementation in rats resulted in significantly higher MDA levels in the liver and kidneys compared with a control diet group.

In the present study, however, the *S. herbacea* L.-supplemented diet decreased the MDA level significantly compared with the group given only the high-fat diet. Similar to our result, many previous studies reported that *S. herbacea* L. supplementation reduced lipid peroxidation of liver in an ovariectomy-induced oxidative stress model (Ha et al., 2006) or STZ-induced diabetic rats (Kim, 2007). Additionally, Han and Kim (2003) reported that adding 10% *S. herbacea* L. to the diet had an antioxidative effect similar to that of adding 1% α -tocopherol and that the antioxidative effect increased in a dose-dependent manner *in vitro*. However, the antioxidative properties of *S. herbacea* L. in the skeletal muscle were found first in this study.

The antioxidative properties are caused by abundant phenolic compounds (Seo et al., 2010) and the high electron-donating ability (Lee and An, 2002) of *S. herbacea* L.

With respect to antioxidative defense mechanisms, oxidative stress is caused by loss of the balance when the free radicals generated exceed the antioxidant defense capacity or the antioxidant defense activity is reduced

(Videla and Fernandez, 1988). SODs play important roles in eliminating ROS and preventing damage by ROS by converting a superoxide anion radical into hydrogen peroxide (McCord and Fridovich, 1969), and GPx is then responsible for the decomposition of hydrogen peroxide to water (Zhang et al., 1996).

The present results showed that the levels of Cu, Zn-SOD, Mn-SOD, and GPx protein in EG were increased significantly versus that of the CG. Previously, Kim (2008) reported that liver GPx activity was increased significantly in both normal and STZ-induced diabetic rats by dietary supplementation with 10% *S. herbacea* L. Similar to our results, it has been reported that intraperitoneally injecting 100 mg/kg of *S. herbacea* L. for 2 months increased the contents of SOD and GPx protein in the livers of rat with ovariectomy-oxidative stress (Ha et al., 2006). Yoshino and Murakami (1998) reported that polyphenolic compounds act as ROS inhibitors due to the formation of inactive Fe²⁺ phenolic complexes through ion chelation.

Thus, *S. herbacea* L. supplementation can have an important role in antioxidative mechanisms due to its high contents of various minerals, including Na, Ca, Mg, Fe, and Zn (Min et al., 2002), and it was suggested that the polyphenolic compounds in *S. herbacea* L. reduced oxidative stress by increasing defensive actions against oxidative damage by undermining free radical activity and increasing antioxidant enzymatic activity.

Conclusions

In summary, the results of this study suggest that *S. herbacea* L. supplementation may increase antioxidant activity by reducing MDA content and increasing the expression of Cu, Zn-SOD, Mn-SOD, and GPx proteins in skeletal muscle in rats.

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