Effects of *Opuntia humifusa* Supplementation on Lipid Peroxidation and SOD Protein Expression in the Liver, Kidney, and Skeletal Muscle of Rats Fed a High-fat Diet

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This study was conducted to investigate the effects of *Opuntia humifusa* supplementation on lipid peroxidation and superoxide dismutase (SOD) protein expression at resting state in various organs of rats fed a high-fat diet. Sixteen Sprague-Dawley male rats, 6 weeks of age, were randomly divided into two groups: a control diet group (CG, n=8) and an experimental diet group (EG, n=8). They were given a high-fat diet (CG) or a diet supplemented with 5% of *O. humifusa* (EG) for 8 weeks. The results showed that the malondialdehyde (MDA) levels of the kidney and the liver were significantly lower in the EG group than in the CG group (p<0.01). In addition, the MDA levels in the skeletal muscle of the EG group tended to be lower than those in the CG group, but this difference was not significant. The Cu, Zn-SOD protein expression in the kidney of the EG group was significantly increased compared with that of the CG group (p<0.01). The Mn-SOD protein expression in the skeletal muscle of the EG group was significantly increased compared with that of the CG group (p<0.01). These results suggest that *O. humifusa* supplementation has antioxidative properties, which are exerted in a specific organ manner, and that it inhibits the action of lipid peroxidation and the expression of SOD in rats fed a high-fat diet.

Key words: Opuntia humifusa, high fat diet, malondialdehyde (MDA), reactive oxygen species (ROS)

Introduction

Reactive oxygen species (ROS) are occurred even in normal cell and these ROS are closely related to diabetes, hypertension and renal dysfunction. In particular, it is well documented that ROS are susceptible to react with polyunsaturated fatty acid (PUFA) and increases lipid peroxidation which induces oxidative damage to cell membrane [20,36].

In addition, previous studies reported that chronic high fat diet supplementation caused increment of ROS in various organs due to induced the metabolic disorder such as obesity, diabetes and hypertension, etc. [1,25]. However, to effectively scavenging ROS and to protect the body against oxidative stress, humans have evolved a highly sophisticated and antioxidative protection system which included antioxidants such as glutathione, flavonoids, and β -carotene, and antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) [11,22,28,35].

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Cactus, which is exist varying of approximately 4,000 kinds in the world, is very adaptable in the semi-arid regions, and is widely used as a supplemental source of carbohydrate and vitamins. In particular, Opuntia humifusa is a member of cactaceae family that has been cultivated in cold environment down below -20°C [10]. However, previous studies reported that Opuntia ficus-indica, which is same member of cactaceae family, has many abundant nutritional factors such as calcium (Ca), sodium (Na), magnesium (Mg), zinc (Zn), and ferrous (Fe) [8,13,32]. Many studies reported that O. ficus-indica have a crucial role for anti-inflammatory [24], lowering blood glucose level [15,34] and anti-ulcerous effect [9]. However, few studies have been reported on physiological role of O. humifusa which is a similar to O. ficus-indica that contains a lot of minerals and vitamins addition to a lot of antioxidants such as flavonoids. In addition, there have not been reported that the protective effects of O. humifusa supplementation on antioxidative acitivities due to high fat diet-induced oxidative damage in vivo in spite of increased radical scavenging activity in vitro [5].

Therefore, we investigated that the effects of *O. humifusa* supplementation on lipid peroxidation and SOD protein expression in various organs of rats fed a high-fat diet.

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Materials and Methods

Experimental animals and diets

Sixteen, 6 weeks old Sprague-Dawley rats weighing 230-250 g were purchased from Samtaco Bio Korea (Hwaseong, S. Korea). The rats were divided into two groups (CG: high-fat diet group, n=8, EG: *O. humifusa* supplemented diet group, n=8), given free access to tap water and diet for 8 weeks, and housed in groups of two per cage under controlled temperature (23±1°C) and relative humidity (50±5%). The light/dark cycle was automatically controlled (alternation 12-h periods) and lighting was begun at 8:00. At the end of the experimental period, the rats were anesthetized with ether after overnight fasting. Liver, kidney and soleus muscle were collected, and all samples were stored at -70°C until analyzed. All studies were approved by Animal Studies Committee of Sunmoon University.

The *O. humifusa*, which was harvested in Asan, Chungnam, were washed cleanly and blended using a blender HMF-3150S (Hanil Electronics, Korea). After blending, the *O. humifusa* was frozen in a freezer at a temperature of -70°C and then freeze-dried in a dryer (Ilshin Co., Korea). Thus, the component analysis of O. humifusa was performed by Eco-Bio Korea (Buchen, Korea), and the result was shown in Table 1. The composition of the high-fat diet was composed of 20% protein, 48% carbohydrate and 20% fat which is modified as previous study [16] was based on AIN-76G and 5% *O. humifusa* diet was made by substituted for a portion of carbohydrate, protein, fiber, and fat components of control diet as a experimental diet.

Biochemical analysis

Malondialdehyde (MDA) levels in liver, kidney and skel-

Table 1. Nutritional contents of *O. humifusa*

Ingredient	Contents
Moisture (%)	2.89
Ash (%)	13.80
Calorie (kcal/100 g)	290.98
Carbohydrate (g/100 g)	46.56
Crude protein (g/100 g)	4.91
Crude fat (g/100 g)	3.06
Fe^{2+} (mg/g)	5.76
Ca^{2+} (mg/100 g)	2931.30
Mg^{2+} (mg/100 g)	1227.90
K^+ (mg/100 g)	2155.50
Na ⁺ (mg/100 g)	30.90
P (mg/100 g)	653.20

etal muscle were determined colorimetrically using thiobarbituric acid according to the method of Buege and Aust method (1978). Briefly, tissues were homogenized with saline and then centrifuged at $8,000\times g$ for 5 min. The supernatant was added to thiobarbituric acid (TBA)-HCl reagent. The mixture was then placed in a boiling water bath for 15 min at $100\,^{\circ}$ C. After cooling on ice rapidly, the protein precipitate was removed by centrifuging at $10,000\times g$ for 5 min, and the absorbance of the upper supernatant fraction was read at 535 nm. MDA values were calculated using molar extinction coefficient, and expressed as μ mol/g weight.

Western blot analysis

Liver, kidney and skeletal muscle were homogenized on ice with a polytron homogenizer in 19 volumes of 20 mmol/l Tris-HCl buffer (pH 7.5) containing 5 mmol/1 EDTA, 2 mmol/1 PMSF, protease inhibitor cocktail (Sigma, St Louis, MO). The homogenates were centrifuged at $1,200 \times g$ for 10 min and then the supernatant was collected and recentrifuged at $10,000 \times g$ for 10 min. The supernatants were used for Cu,Zn-SOD protein expression. The pellets were resuspended in homogenizing buffer and used for Mn-SOD protein expression. Protein concentrations were determined by the use of a Bradford reagent from Bio-Rad, with bovine serum albumin as the standard. All protein extraction procedures were conducted at 4°C. An aliqout of tissue extract containing 20 µg of protein was mixed with an equal volume of Laemmli buffer, heated at 100°C in heating block for 5 min, and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis along with a mixture of molecular-weight standards from Bio-Rad (Hercules, CA). After electrophoresis, the proteins were transferred to a PVDF (polyvinylidene difluoride) membrane (Bio-Rad) in a Mini Transfer-Blot electrophoretic Transfer cell (Bio-Rad). After treating with blocking buffer (PBS containing 10% Skim milk) for 90 min at room temperature, the membranes were incubated with primary polyclonal antibodies for 2 hr at room temperature. These included anti-SOD1 (Santa Cruz, CA) and anti-SOD2 (Santa Cruz, CA). The antibodies were raised in goat against the rat isoforms of the above proteins. The membrane was then incubated with horseradish peroxidase-conjugated secondary antibodies, which were anti-goat IgG and anti-rabbit IgG (Santa Cruz, CA), as appropriate, for 1 hr at room temperature. The target proteins were detected by an enhanced chemiluminsce kit (GE, USA). The films were photographed and the protein bands of interest

were quantified with the band analyzer software (Bio-Rad, USA).

Statistical analysis

All data were analyzed using SPSS software (version 16.0 for Windows). Data are expressed as the mean \pm SE, and values were analyzed by the independent samples t-test. Significance was defined as α =0.05.

Results and Discussion

As shown in Fig. 1, the MDA contents of the EG group were significantly lower than that of the CG group in kidney and liver (p<0.01; Fig. 1A and Fig. 1B, respectively). Also, the MDA level in the soleus muscle of the EG group was tended to have lower than that of the CG group, but no significant difference. Many studies reported that high-fat diet induces MDA levels in tissue of rat compared to normal diet [1,2]. Of these, Noeman et al. (2011) reported that MDA levels of kidney and liver in high fat diet rats were significantly increased compared to normal diet rats. In particular, lipid peroxidation leading to renal injury is occurred via abnormal renal lipid accumulation that induces imbalance between lipogenesis and lipolysis and furthermore leading to systemic metabolic abnormalities [18]. In addition, dyslipidemia with high-fat diet also induces hepatic lipid peroxidation due to the increased triacylglycerol and increased influx of excess the non esterified fatty acid (NEFA) into the liver [12]. In fact, the blood triglyceride concentration of CG group was significantly increased compared to EG group (36.0 \pm 2.69 mg/dl vs 18.0 \pm 3.07 mg/dl, p<0.05)

However, in this study, we found that MDA levels of kidney and liver were significantly decreased by the *O. humifusa*

supplementation and similar trend are shown in skeletal muscle. As written in above, it is well known that *O. humifusa* contains a lot of antioxidant such as a quercetin, etc [5,8]. In fact, Cho et al. (2006) reported that *O. humifusa* treatment showed potential ROS scavenging effects in *in vitro* and these effects are may be due to quercetin and its derivatives of *O. humifusa*. However, physiological role of antioxidative capacity of *O. humifusa* are yet controversial *in vivo*

As shown in Fig. 2, the renal Cu,Zn-SOD protein expression of EG group was significantly higher than that of the CG group (p<0.01; Fig. 2A). Also, the hepatic and muscle Cu,Zn-SOD protein expression of the EG group was tended to have higher than that of the CG group in spite of no significant differences. SOD is one of the antioxidant enzymes that superoxide radicals reduce to H₂O₂ and water and used as a oxidative stress marker in cell [29]. Many studies reported that quercetin present in O. humifusa which is contains a number of phenolic hydroxyl groups that have strong antioxidant activity [21,33] than other antioxidant nutrients such as vitamin C, E and β-carotene [27]. In fact, quercetin has a powerful antioxidative capacity. For example, Renugadevi and Prabu (2010) reported that rats received daily 50 mg/kg BW of quercetin treatment for 4 weeks increased SOD activity and vitamin C and vitamin E levels in kidney. Similar to our results, Liu et al. (2010) reported that the quercetin treatment for 10 weeks in lead-induced hepatic damaged rats increased Cu, Zn-SOD activity of kidney. In the present study, O. humifusa supplementation effects on Cu,Zn-SOD protein expression of kidney due to flavonoids such as quercetin and its derivatives. However, Cu,Zn-SOD protein expressions of liver and skeletal muscle were not shown significant effects. Previous studies reported that concentrations of quercetin are differ with the various

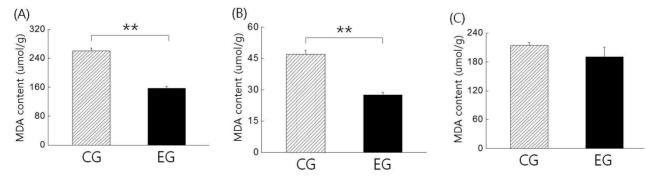


Fig. 1. Effect of *O. humifusa* supplementation on the MDA contents in organs (A, kidney; B, liver, C, soleus muscle). CG, High fat diet group; EG, *O. humifusa* supplemented group. *Significantly different between CG and EG groups; Values are mean±SE; **p<0.01 are compared with CG group.

tissues even though oral supplemented in rats or pigs [3,7]. Bieger et al (2008) reported that the highest levels of quercetin are shown in kidney and relatively lowered in liver and skeletal muscle fed a 50 mg/kg/day of quercetin for 4 weeks in rats. Therefore, increased Cu,Zn-SOD protein expression maybe affected by the increased quercetin level in the kidney tissue by the supplementation of *O. humifusa*.

As shown in Fig. 3, the Mn-SOD protein expression of EG group on the soleus muscle was significantly higher than that of the CG group (p<0.01; Fig. 3C). And the Mn-SOD protein expression in the kidney and liver of the EG group was tended to have higher than that of the CG group, but no significant difference.

Excess dietary fat induced obesity maybe affect on the development of mitochondrial dysfunction, as supported by a recent study showing down regulation of genes involved with oxidative phosphorylation and mitochondrial biogenesis in response to high-fat feeding [30]. Sreekumar et

al. (2002) reported that the high-fat diet decreased antioxidant enzyme activities, result in decreased Cu,Zn-SOD and Mn-SOD mRNA levels of skeletal muscle. However, Davis et al. (2008) reported that quercetin feeding of 25 mg/kg for 7 days effect on increased mitochondrial function through increased in sirtuin 1 (SIRT1) and peroxisome proliferator-activated receptor- γ coactivator (PGC-1a) gene expression associated with mitochondrial biogenesis and increased mitochondrial DNA (mtDNA) in skeletal muscle and brain. In the present study, flavonoids such as quercetin in *O. humifusa* may be effect on mitochondrial function simultaneously with increased Mn-SOD protein expression in skeletal muscle.

From these results, it was suggested that *O. humifusa* supplementation has a positive effects on antioxidative properties in a specific organ manner that inhibitory action of lipid peroxidation and expression of SOD in rats fed a high-fat diet.

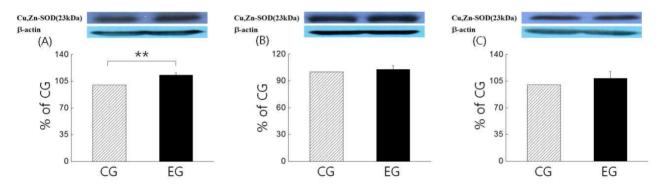


Fig. 2. Effect of *O. humifusa* supplementation on Cu/Zn-SOD protein expression tin organs (A, kidney; B, liver, C, soleus muscle). CG, High fat diet group; EG, *O. humifusa* supplemented group. *Significantly different between CG and EG groups; Values are mean±SE (% of CG); *** p<0.01 are compared with CG group.

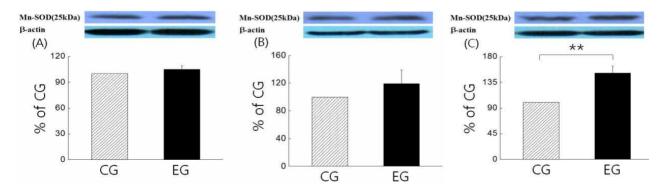


Fig. 3. Effect of O. humifusa supplementation on Mn-SOD protein expression in organs (A, kidney; B, liver, C, soleus muscle). CG, High fat diet group; EG, *O. humifusa* supplemented group. *Significantly different between CG and EG groups; Values are mean±SE (% of CG); *** p<0.01 are compared with CG group.

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초록: 손바닥 선인장 보충이 고지방식이 흰쥐의 간장, 신장 골격근에 지질괴산화와 SOD단백질 발현에 미치는 영향

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본 연구는 안정 시 고지방식이 흰쥐의 신장, 간장 그리고 골격근에서 지질과산화와 SOD 단백질 발현에 손바닥선인장 보충이 미치는 효과에 대하여 연구하였다. SD계 수컷 흰쥐 16마리를 무작위로 대조군(CG, n=8)과 실험군(EG, n=8)으로 분류하였다. 8주 동안 대조군은 고지방식이를 부하하였으며, 실험군은 5% 손바닥선인장을 보충식이하였다. 본 실험결과, 간장과 신장의 MDA 농도는 EG군이 CG군에 비해 유의하게 낮게 나타났다(p<0.01). 또한 골격근의 MDA 농도도 EG군이 CG군에 비해 높은 경향이 나타났으나, 통계적으로 유의한 차이는 나타나지 않았다. Cu,Zn-SOD 단백질 발현은 신장에서EG군이 CG군에 비해 유의하게 증가하였다(p<0.01). Mn-SOD 단백질 발현은 골격근에서 EG군이 CG군에 비해 유의하게 증가하였다(p<0.01). 이상의 결과로부터 손바닥선인장 보충이고지방식이 흰쥐의 지질과산화 억제작용과 SOD단백질 발현이 기관특이적인 반응의 항산화 효과가 있는 것으로나타났다.