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Saengshik, a Formulated Health Food, Prevents Liver Damage in CCl_4-Induced Mice and Increases Antioxidant Activity in Elderly Women

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ABSTRACT *Saengshik* is a Korean noncooked food made with of more than 30 different whole gains, vegetables, fruits, mushrooms, and seaweeds. All of these ingredients are frozen and dried to minimize the loss of nutrients. *Saengshik* has become popular among health-conscious people in the Republic of Korea. The study aims to investigate antioxidant effects of *Saengshik* by *in vivo* and human experiments. In *in vivo* tests, mice were fed *Saengshik* for 4 weeks, and oxidative damage was induced by CCl_4. Then the effects of *Saengshik* on oxidative damage were examined. It was found that plasma lipid hydroperoxide and protein oxidative damages were significantly suppressed and antioxidants, glutathione, and thiol groups were increased. The activity of the antioxidant enzyme superoxide dismutase was increased, and the level of glutamate pyruvate transaminase was decreased. In a human study, elderly people were given *Saengshik* for 24 weeks, and changes in antioxidant defense of the body were examined. Antioxidant activities in plasma were enhanced, although the difference was not significant. Therefore, it is expected that *Saengshik* is effective at removing oxidants from body tissues, preventing oxidative damage, and eventually boosting the antioxidant capacity of the body.

KEY WORDS: • ***antioxidant activity*** • ***CCl_4*** • ***elderly women*** • ***raw food*** • **Saengshik** • ***uncooked food***

INTRODUCTION

According to advances in technologies and improving standard of living, the average life expectancy has greatly increased, leading to an increase in the aged population in most countries around the world. However, there are chronic diseases such as cancer, cardiovascular diseases, and heart diseases not curable even in today's medical context. In particular, it is estimated that 88% of the elderly population 65 years of age or above suffer from at least one disease.[1] Several studies have revealed that the incidence of chronic regressive diseases is closely related to reactive oxygen species causing oxidative damage.[2] These reactive oxygen species are known to occur as a result of oxidative stress, but they are generated naturally in body metabolism. As many studies have reported an important impact of dietary habits on the aging process and disease, people have become interested in healthy and functional food in a move to prevent diseases and improve nutritional status.[3] As a result, a variety of functional foods or nutrient supplements, to improve health and substitute for a regular meal, have hit the market. *Saengshik* is particularly popular among people with a busy lifestyle because of its well-balanced nutrients and easy-to-eat convenience. *Saengshik*, which includes 30–50 different whole grains, beans, seeds, vegetables, seaweeds, and mushrooms, is manufactured by freeze-drying to avoid loss of nutrients such as vitamins, minerals, dietary fiber, and phytochemicals.[4,5]

Previous studies reported beneficial effects of *Saengshik* intake on various health problems such as diabetes, obesity, blood composition of women, fatty liver, and hyperlipidemia. These studies also proved positive effects of *Saengshik* intake on physical strength, detoxification, antioxidation in an imbalanced diet model, and blood plasma mineral concentration.[6–14] However, there are not many studies providing effects of *Saengshik* intake on liver damage in CCl_4-induced rats and antioxidant activity in elderly women. The present study aimed to investigate the antioxidant effects of *Saengshik* intake on damage induced in liver tissue in an *in vivo* animal experiment and in humans on the health of elderly people.

MATERIALS AND METHODS

In vivo *animal experiment*

Experimental diet and animals. ICR mice, 4 weeks old, were received from Daehan Biolink Co., Ltd. (Eumseong,

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Republic of Korea) and held for 1 week to acclimate to the laboratory prior to the experiment. Animals were divided into three groups ($n = 10$): Control group (normal diet + no CCl_4 injection), CCl_4 group (normal diet + CCl_4 injection), and 30% S group (30% *Saengshik* diet + CCl_4 injection).

Compositions of experimental diets are presented in Table 1. The ingredients of basal diet were purchased from Dyets Inc. (Bethlehem, PA), and *Saengshik* was offered by Erom Co., Ltd. (Seongnam, Republic of Korea). Ingredients and nutrient contents of *Saengshik* are presented in Tables 2 and 3. The nutrient contents of *Saengshik* were analyzed by the Korea Health Industry Development Institute (Seoul, Republic of Korea). To prevent any imbalance of nutrients that might be possible when experimental diet containing 30% *Saengshik* was made, we adjusted nutrient contents and calorie counts in control and experimental diets. All rats were fed these diets for 4 weeks.

Oxidative damage in animals and sample collection. After 4 weeks of feeding, CCl_4 in olive oil (a ratio of 1:1) was injected peritoneally at a concentration of 1 mg/kg of body weight. The CCl_4 group and the 30% S group received two CCl_4 injections: the first injection at 3 days and the second injection at 1 day prior to sacrifice. Those animals that had received a CCl_4 injection were not fed for 18 hours before sacrifice. Animals were anesthetized using ether, and then blood samples from heart and liver tissues were obtained. Collected blood was centrifuged to separate plasma for testing sample. Liver tissue was treated with 9× phosphate-buffered saline containing 0.5% Triton X-100 to produce homogenized solution and then used as the test sample.

Table 1. Composition of the Experimental Diets

Ingredient	*Control (%)*	*30% Saengshik (%)*
Casein	20.00	18.13
Cornstarch	39.75	22.00
Dextrinized cornstarch	13.20	7.28
Sucrose	10.00	10.00
Cellulose	5.00	5.00
Soybean oil	7.00	5.80
Mineral mixture[a]	3.50	3.50
Vitamin mixture[b]	1.00	1.00
l-Cysteine	0.30	0.30
Choline bitartrate	0.25	0.25
Saengshik	—	30.00
Nutrients		
Carbohydrate (%)	67.9	67.9
Protein (%)	20.3	20.3
Fat (%)	7.0	7.0
Metabolic energy (kcal/100 g)	416.0	416.0

[a]AIN93G mineral mix contained (in g/kg of mix): calcium carbonate, potassium phosphate monobasic, 196; potassum citrate monohydrate, 70.78; sodium chloride, 74.00; potassium sulfate, 46.6; magnesium oxide, 24; ferric citrate, 6.06; zinc carbonate, 1.65; manganese carbonate, 0.63; copper carbonate, 0.3; potassium sulfate · 12 H_2O, 0.275; ammonium paramolybdate · 4 H_2O, 0.00795; sodium metasilicate · 9 H_2O, 1.45; chromium potassium sulfate · 12 H_2O, 0.275; ammonium vanadate, 0.0066; lithium chloride, 0.0174; boric acid, 0.08145; sodium fluoride, 0.0635; nickel carbonate, 0.0318; powdered sucrose, 221.026.

[b]AIN93G vitamin mix contained (in g/kg of mix): nicotinic acid, 3.0; calcium pantothenate, 1.6; pyridoxine-HCl, 0.7; thiamine-HCl, 0.6; riboflavin, 0.6; folic acid, 0.2; biotin, 0.02; vitamin B12 (0.1% in mannitol), 2.5; vitamin E (500 IU/g), 15.0; vitamin A (500,000 IU/g), 0.8; vitamin D3 (400,000 IU/g), 0.25; vitamin K1 (phylloquinone), 0.075; powdered sucrose, 974.655.

Table 2. Raw Materials of *Saengshik*

Brown rice
Glutinous millet
Sorghum
Prosomillet
Soybean
Black sesame
Black rice
Barley
Kale
Carrot
Burdock
Pumpkin
Angelica utills
Cabbage lotus root
Spinach
Chlorella
Lentinus edodes
Mugwort
Pine needle
Laver
Brown seaweed
Sea tangle
Yeast
Bifidobacterium longum
Fructooligosaccharide
Lactoferin
Rose petal extract

Analysis of blood plasma and oxidative damage in liver. Malondialdehyde and thiobarbituric acid, secondary metabolites of lipid hydroperoxidation in plasma and liver tissue, were measured according to the method of Yagi.[15] Protein oxidative damage was measured quantitatively and compared with that of a standard substance, glycine, using fluorescamine.[16]

Analysis of antioxidant defense system. Glutathione concentration was determined by measuring the reaction of dithiodinitrobenzoic acid with the thiol group. Total thiol group concentration was measured using 6,6-dithiodinicotinic acid.[17] The activities of enzymatic antioxidant defense system *in vivo* (superoxide dismutase [SOD], catalase [CAT], glutathione peroxide [GPx], and glutathione reductase [GR]) were measured in liver tissue. The activity of SOD resulting in 50% inhibition of autooxidation of pyrogallol is defined as 1 unit.[18] CAT enzyme activity was determined according to the method of Aebi,[19] and 1 unit is equal to the amount of H_2O_2 released per minute. GR ac-

TABLE 3. NUTRIENT CONTENTS IN *SAENGSHIK*

Nutrient	*Contents*
Energy (kcal)	392.6
Moisture (%)	1.9
Carbohydrate (%)	80.9
Protein (%)	11.4
Fat (%)	2.6
Na (mg/100 g)	176.5
Fe (mg/100 g)	3.54
Zn (mg/100 g)	7.83
Ca (mg/100 g)	600.0
Vitamin A (μg of RE/100 g)	368.7
Vitamin B1 (mg/100 g)	1.8
Vitamin B2 (mg/100 g)	0.4
Vitamin B6 (mg/100 g)	0.4
Vitamin C (mg/100 g)	43.5
Vitamin D3 (mg/100 g)	21.6
Vitamin E (mg/100 g)	5.1
Folic acid (mg/100 g)	1.9
Niacin (mg/100 g)	9.9

RE, retinol equivalent.

tivity was determined by measuring the amount of reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH) using NADPH as the hydrogen donor. GPx enzyme activity was determined by measuring the amount of NADPH generated to catalyze the reduction of GSH to GSSG,[20] and 1 unit will reduce 1.0 μmol of GSSG/minute at pH 7.6 at 25°C.

Human study

Subjects. A total of 33 women over 65 years old living in Seoul and five other Korean metropolitan cities participated in the study. The mean age of subjects was 76.2 ± 5.9 years.

Experimental methods. *Saengshik* was offered by Erom Co., Ltd. Subjects were instructed to take 40 g of *Saengshik*, one serving size, once a day for 24 weeks and ate their three regular meals as usual. Their intake of *Saengshik* was monitored by telephoning them regularly. *Saengshik* was supplied every 4 weeks after confirmation that the subjects ate all the *Saengshik* previously supplied. Anthropometric measurements and fasting blood tests were undertaken before and after *Saengshik* supplementation.

Anthropometric measurement and blood pressure measurement. All subjects underwent measurements of height, weight, body fat, and blood pressure. Height was measured in the standing position keeping the waist straight. Weight and body fat were measured using a digital body fat measurement (UM-015, Tanita Co., Tokyo, Japan). Blood pressure (systolic and diastolic) was measured using an electronic blood pressure measurement device (UA-767, A&D Co., Tokyo).

Blood analysis. All venous blood samples (10 mL) were collected in the early morning after the subjects had fasted for 12 hours. Four milliliters of blood was collected in venipuncture tubes containing EDTA for hemocyte analysis such as erythrocyte, hemoglobin, and hematocrit. Hemocyte analysis was conducted using a blood cell analyzer on the same day after collection of blood sample. Six milliliters of blood was left at room temperature for about an hour and centrifuged at 1,010 *g* at 4°C for 15 minutes to separate serum. Total proteins, albumin, total cholesterol, high-density lipoprotein-cholesterol, low-density lipoprotein-cholesterol, and triglyceride in serum were measured. Blood sugar level was measured using an automatic blood sugar level measuring device (Accu-Chek® Softclix, Roche, Mannheim, Germany) when venous blood was collected. Analysis of blood markers in serum was commissioned by Green Cross Corp. (Yongin, Republic of Korea).

Measurement of antioxidant status. Plasma total antioxidant status (TAS) was measured using a TAS kit (Randox Laboratories, Ltd., Crumlin, UK). This involved mixing 20 μL of calibrator (1.79 mmol/L 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) or sample with 1 mL of chromogen [6.1 μmol/L metmyoglobin and 610 μmol/L 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)] and incubating at 37°C for 3 minutes. Initial absorbance was read at 600 nm (GENios, Tecan Group Ltd., Salzburg, Austria). Then, 200 μL of substrate (250 μmol/L hydrogen peroxide) was added to calibrator and sample and incubated at 37°C for 3 minutes. Then absorbance was read at 600 nm. The change in absorbance value for samples relative to the change in absorbance of the calibrator was then to calculate the TAS in all samples.

Statistical analysis

An SPSS Win version 8.1 program (SPSS, Chicago, IL) was used to calculate mean and standard deviations. One-way analysis of variance and Duncan's multiple comparison test were performed to determine differences among

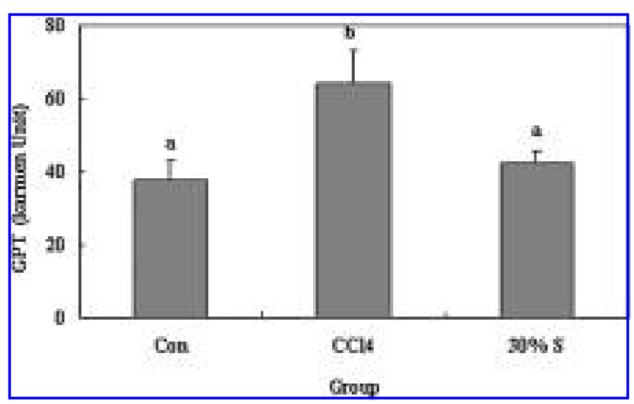

FIG. 1. Protective effects of *Saengshik* on CCl_4-induced liver damage in mouse liver: Con (normal diet), CCl_4 (normal diet + CCl_4 injection, i.p.), and 30% S (30% *Saengshik* diet + CCl_4 injection, i.p.). Means with the same letter are not significantly different at $P < .05$.

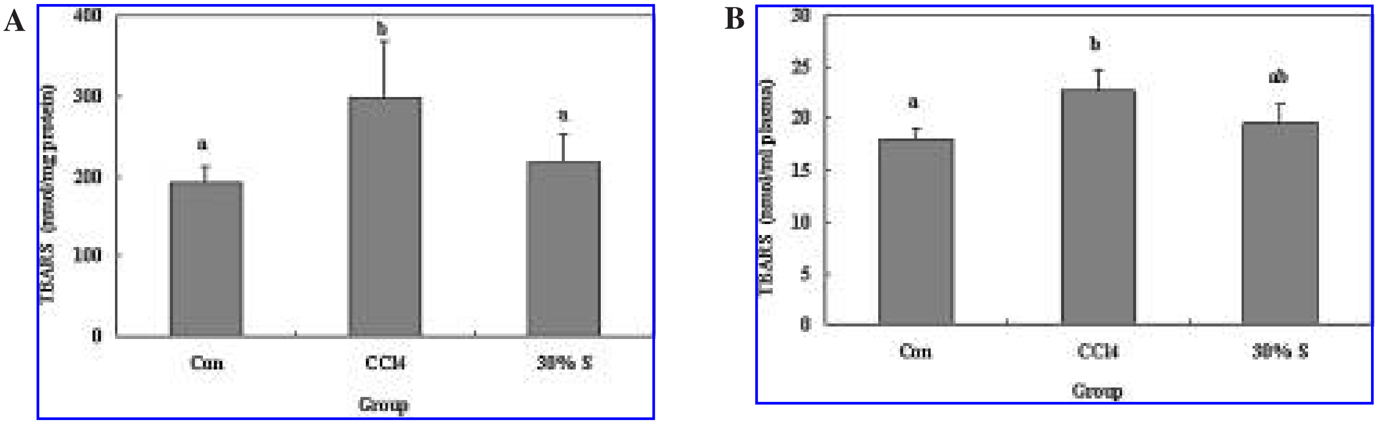

FIG. 2. Protective effects of *Saengshik* on CCl_4-induced oxidative damage in (**A**) mouse liver and (**B**) plasma: Con (normal diet), CCl_4 (normal diet + CCl_4 injection, i.p.), and 30% S (30% *Saengshik* diet + CCl_4 injection, i.p.). Means with the same letter are not significantly different at $P < .05$. TBARS, thiobarbituric acid-reactive substances.

animal groups. The level of $P < .05$ was considered statistically significant. Student's t test was performed to the difference among subjects before and after *Saengshik* intake.

RESULTS

In vivo *experiment*

Measurement of liver function marker. CCl_4 injection increased the glutamate pyruvate transaminase (GPT) level in plasma, whereas CCl_4 injection of *Saengshik*-fed rats decreased the GPT level. It was found that mixed diet containing *Saengshik* (30%) reduced liver toxicity caused by increased activity of GPT after CCl_4 injection (Fig. 1).

Effects of oxidative damage suppression in plasma and liver. Lipid hydroperoxide appearing in liver tissue after CCl_4 injection was measured (Fig. 2). Increased lipid hydroperoxide levels in plasma and liver tissue of the CCl_4 group confirmed that oxidative damage was induced. When CCl_4 was injected into mice that ate *Saengshik* (30%) in their diet for 4 weeks, levels of lipid hydroperoxide were 30% less in liver tissue and 15% less in plasma, compared with those of the CCl_4 group. Protein degradation was measured to assess protein oxidative damage (Fig. 3). Like lipid hydroperoxide, protein oxidative damage increased as a result of CCl_4 injection. However, protein degradation was significantly suppressed in the *Saengshik* group.

Analysis of antioxidant defense system. Changes were observed in the concentration of glutathione and the thiol group, which play a pivotal role in the nonenzyme antioxidant defense system (Fig. 4). The concentrations of glutathione and the thiol group were reduced following CCl_4 injection. However, higher amounts of glutathione and the thiol group were observed in the *Saengshik* group, compared with the CCl_4 group.

Changes in antioxidant enzyme activities in liver tissue were examined after CCl_4 injection (Fig. 5). SOD activity, which is known to decrease the scavenger reaction of superoxide ($O^{2\bullet}$), was reduced by 70% in the CCl_4 group, compared with that of the control group. However, the *Saengshik* group showed an SOD activity level similar to that of the control group, leading to the conclusion that 30% *Saengshik* helped maintain the same level of SOD activity despite CCl_4 injection.

CAT activity was also reduced after CCl_4 injection, although there were no significant differences among the groups. CAT activity increased in the *Saengshik* group, but there was no significant statistical difference, compared with CAT activity of the CCl_4 group. Increased activities of GPx and GR as a result of CCl_4 injection declined significantly in the *Saengshik* group.

Human study

Effects of Saengshik *on body measurements*. Body measurements of subjects (Table 4) showed body weight of 54.0 kg, body mass index of 24.6 kg/m^2, and systolic blood pres-

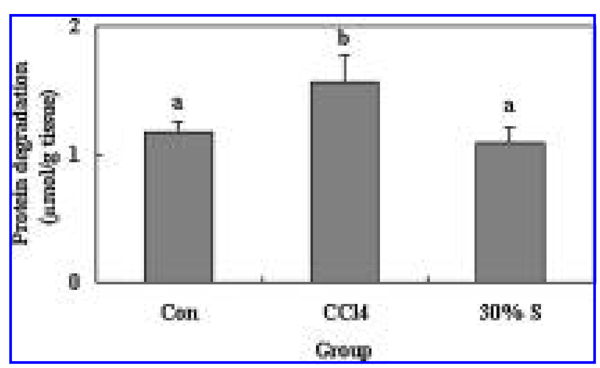

FIG. 3. Protective effects of *Saengshik* on CCl_4-induced protein oxidative damage in mouse liver: Con (normal diet), CCl_4 (normal diet + CCl_4 injection, i.p.), and 30% S (30% *Saengshik* diet + CCl_4 injection, i.p.). Means with the same letter are not significantly different at $P < .05$.

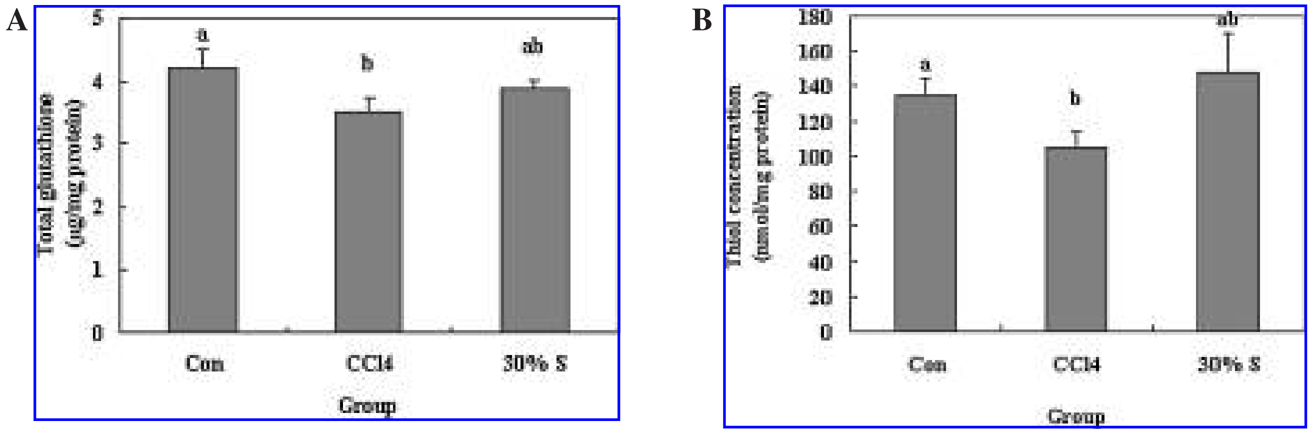

FIG. 4. Effects of *Saengshik* on CCl_4-induced change of (**A**) total glutathione and (**B**) total thiol group contents in mouse liver: Con (normal diet), CCl_4 (normal diet + CCl_4 injection, i.p.), and 30% S (30% *Saengshik* diet + CCl_4 injection, i.p.). Means with the same letter are not significantly different at $P < .05$.

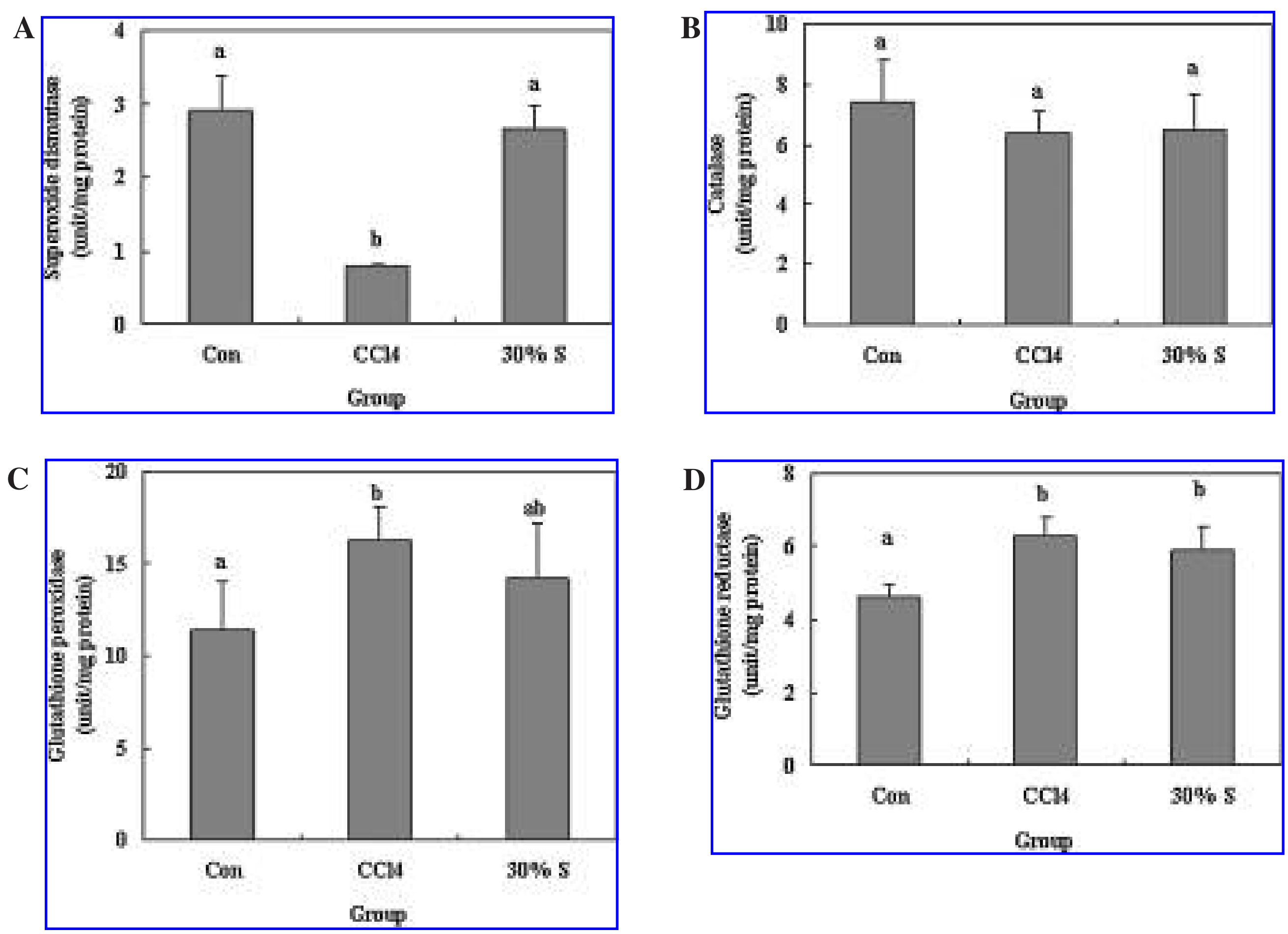

FIG. 5. Effects of *Saengshik* on CCl_4-induced change of the antioxidant enzymes (**A**) SOD, (**B**) CAT, (**C**) GPx, and (**D**) GR in mouse liver: Con (normal diet), CCl_4 (normal diet + CCl_4 injection, i.p.), and 30% S (30% *Saengshik* diet + CCl_4 injection, i.p.). Means with the same letter are not significantly different at $P < .05$.

TABLE 4. ANTHROPOMETRIC VALUES OF SUBJECTS BEFORE AND AFTER *SAENGSHIK* SUPPLEMENTATION

	Saengshik *supplementation*	
	Before	*After 24 weeks*
Body weight (kg)	54.0 ± 8.5	53.9 ± 8.3
Body mass index (kg/m^2)	24.6 ± 3.6	24.5 ± 3.6
Systolic pressure (mm Hg)	144.0 ± 23.3	145.7 ± 23.7
Diastolic pressure (mm Hg)	80.2 ± 10.4	81.0 ± 9.0

Data are mean ± SD values.

All the measured variables were not significantly different at $P <$ .05 by paired t test between before and after 24 weeks of *Saengshik* supplementation.

sure of 144.0 mm Hg and diastolic blood pressure of 80.2 mm Hg before *Saengshik* supplementation. There were no significant differences in body measurements and blood pressure between pre- and post-*Saengshik* supplementation.

Blood analysis. Blood samples of subjects were collected before and after 24 weeks of *Saengshik* supplementation and analyzed as presented in Table 5.

Hemoglobin, plasma proteins, and lipid status showed normal ranges, and no significant differences were found in blood indicators before and after *Saengshik* intake. Seven of the subjects showed a higher level of blood triglyceride than normal, but they returned to normal blood triglyceride levels after 24 weeks of *Saengshik* intake.

TAS. TAS of subjects was measured to examine effects of *Saengshik* on antioxidant capacities in human body (Table 6). There were no significant differences in TAS between before and after *Saengshik* supplementation. However, TAS increased by 23.4% at 12 weeks and by 36.2% at 24 weeks after *Saengshik* intake compared with baseline.

TABLE 5. BLOOD-RELATED PARAMETERS IN SUBJECTS BEFORE AND AFTER *SAENGSHIK* SUPPLEMENTATION FOR 24 WEEKS

	Saengshik *supplementation*	
	Before	*After 24 weeks*
RBCs (10^6/mm^3)	3.9 ± 0.5	3.9 ± 0.5
Hb (g/dL)	11.3 ± 1.5	11.5 ± 1.8
Hct (%)	35.1 ± 4.9	35.6 ± 5.0
Total protein (g/dL)	7.0 ± 0.5	7.2 ± 0.6
Albumin (g/dL)	3.8 ± 0.4	4.1 ± 0.3
Cholesterol (mg/dL)	189.9 ± 47.7	189.5 ± 38.3
TG (mg/dL)	144.9 ± 98.3	140.4 ± 75.3
HDL-C (mg/dL)	50.6 ± 10.2	52.0 ± 11.0
LDL-C (mg/dL)	110.4 ± 44.9	112.5 ± 38.3

Data are mean ± SD values. RBCs, red blood cells; Hb, hemoglobin; Hct, hematocrit; TG, triglyceride; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol.

All the measured variables were not significantly different at $P <$ 0.05 by paired t test between before and after 24 weeks of *Saengshik* supplementation.

DISCUSSION

A large number of epidemiological studies have reported whole grains, vegetables, and fruit help reduce the risk of chronic diseases such as cancer and heart diseases, citing vitamin, minerals, fiber, and phytochemicals as main contributors. These nutrients have various beneficial effects; in particular, their protective effects against oxidative stress and damage are well known. Oxidative stress and damage can trigger the occurrence of various diseases and affect the progress of disease. So a diet effective for suppressing oxidative stress and damage will also help prevent and cure diseases.[21,22] As mentioned above, *Saengshik* consists of frozen-dried whole grains and a variety of vegetables. So it has benefits that can avoid loss of nutrients by heating and possesses various physiological activities. In a previous study the antioxidant activity of *Saengshik* was assessed in an unbalanced diet model, and the change of antioxidant defense system in this model was induced by a deficiency of minor nutrients. However, this model showed a limitation that does not lead to significant change in the antioxidant defense system by the lack of minor nutrients.[14] There have been a few studies of the effects of *Saengshik* on antioxidant capacities of the body. The present study aimed to investigate the antioxidant effects of *Saengshik* diet on oxidative damage induced by CCl_4 in animals and with elderly women.

In the liver, CCl_4 is metabolized by cytochrome P450-dependent monooxygenase systems followed by its conversion to the reactive metabolite, trichloromethyl free radical ($\cdot CCl_3$). $\cdot CCl_3$ causes consecutive lipid peroxidation of the cell membrane and endoplasmic reticulum. $\cdot CCl_3$ free radicals undermine cell functions in contact with components such as nuclear acids, proteins, and lipids, and they cause fatty liver through interruption of lipoprotein release. Sometimes $\cdot CCl_3$ free radicals react with O_2 and are oxidized to trichloromethylperoxy free radical ($\cdot Cl_3COO$), which in turn initiates lipid peroxidation and results in severe damage to cell membrane functions and eventually the liver. Still, cy-

TABLE 6. PLASMA ANTIOXIDATIVE CAPACITY OF SUBJECTS BEFORE AND AFTER *SAENGSHIK* SUPPLEMENTATION

Saengshik *supplementation*	*TAS (nmol/L)*
Before	0.84 ± 0.4
After	
12 weeks	1.03 ± 0.3
24 weeks	1.14 ± 0.6

Data are mean ± SD values.

All the measured variables were not significantly different at $P <$ .05 by paired t test between before and after 24 weeks of *Saengshik* supplementation.

tosolic enzymes flow into the blood and boost activities of liver enzymes in the blood.[23,24] In the present study, the *Saengshik* group showed significantly lower levels of GPT activity than the CCl_4 group. At the same time, thiobarbituric acid-reactive substances in plasma and liver tissue and oxidative protein damages were significantly suppressed in the *Saengshik* group. These results are consistent with the findings of earlier studies in which the injection of carotenoid and phenolic constituents of vegetables and fruits into animals with CCl_4-induced liver damage suppressed glutamic oxaloacetic transaminase and GPT activities and lipid hydroperoxide concentration.[25,26] Jung *et al*.[27] also reported a significant decrease in lipid hydroperoxide concentration in damaged liver tissue of rats after injection of *Angelica keiskei* juice. It is therefore assumed that *Saengshik* intake helps prevent or suppress oxidation and blocks eventual damage to cell membrane. As a result, GPT release, along with generation of lipid hydroperoxide and oxidative protein, can be prevented.

The antioxidant defense system exists to remove oxidants generated during oxidation in the human body. The enzymatic antioxidant defense systems include SOD, CAT, and GPx, and the nonenzymatic antioxidant defense systems include glutathione, vitamin A, vitamin C, vitamin E, and β-carotene.[28] It has been reported that CCl_4-induced oxidants increase oxidative stress and influence on the balance of the antioxidant defense system within the body.

SOD is an enzyme that catalyzes the conversion of superoxide anion into hydrogen peroxide and protects cells from free radicals. It was found that SOD activity decreased in animals when CCl_4 was injected.[29,30] CAT is an enzyme that catalyzes the decomposition of hydrogen peroxide into water and oxygen. CAT activity was also decreased following CCl_4 injection.[29] In the present study, the CCl_4 group showed significantly lower levels of SOD and CAT activities than the control group. The level of SOD activity in the *Saengshik* group recovered to a value similar to that of the control group, and the CAT activity increased, but the difference was statistically significant.

GPx and GR are enzymes that catalyze the reduction of lipid peroxidation.[31] GPx uses GSH as a substrate to remove H_2O_2.[32] GPx activity increased following CCl_4 injection.[33] GR catalyzes the conversion of GSSG to GSH, the substrate of GPx.[34] It is assumed that increased GPx activity requires more substrate, leading to an increase in GR. The present study found an increase in both GPx and GR after CCl_4 injection and that *Saengshik* intake helped in the recovery to normal levels of GPx and GR by reversing increased activities. The concentrations of nonenzyme components of antioxidant defense, glutathione, and thiol groups in liver tissue decreased following CCl_4 injection but increased back to normal levels in the *Saengshik* group. It is assumed that the activities of enzymes increased by CCl_4 increase the use of substrates such as GSH for scavenging reactive oxygen.

In this study, we found that *Saengshik* intake influences activities of antioxidant defense enzymes as well as nonenzyme components and removes and suppresses excess oxidants. Then we studied whether consumption of *Saengshik* was associated with antioxidant activity in the human body.

Oxidative stress occurs when there is an excess of oxidants and the balance of the antioxidant defense system of the body is disturbed. Oxidative stress may cause Alzheimer's disease, Parkinson's disease, and cataracts among elderly people. It is therefore necessary to destroy oxidants accumulated within the body by activating the antioxidant defense system.[35] A variety of phytonutrients such as vitamins and flavonoids play an important role in preventing chronic diseases.[36,37] In this study, elderly women showed an increase of TAS after 24 weeks of *Saengshik* supplementation, but the difference was not significant. However, it was positively correlated with the period of *Saengshik* supplementation. Therefore, it is expected that *Saengshik* supplementation for a long period plays an important role in maintaining elderly people's health. The increase of the body's antioxidant capacity among subjects is support for the findings that components of *Saengshik* are various and *Saengshik* is a whole food. Other studies support the results of our study. The study of Cao *et al*.[38] showed that flavonoid rich-diet increased plasma antioxidant levels in elderly women. Liu[39] reported that antioxidant effects are elevated more when combinations of different fruits and vegetables are taken than when a fruit or vegetable is taken alone and that a whole-food diet plays an important role in maintaining health because it contain numerous phytochemicals differing in molecular sizes, polarity, and solubility.

Therefore, it has been shown that *Saengshik* has a positive effect on the antioxidant defense system by scavenging oxidants in an *in vivo* animal model and with an increase of total antioxidant activity in the human body. It is concluded that nutritional properties derived from the more than 30 different ingredients of *Saengshik* serve as stimulants of enzyme activities and scavengers of oxidants, resulting in elevated antioxidant capacities of the body. It is likely that *Saengshik* intake would help prevent chronic diseases and slow down the aging process. Further studies are needed to investigate the effects of *Saengshik* on antioxidant defense mechanisms.

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