DOI:10.1021/jf103938e

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Chemical Characterization of the Avenanthramide-Rich Extract from Oat and Its Effect on D-Galactose-Induced Oxidative Stress in Mice

Yi Ren,[†,‡] Xiushi Yang,[†,§] Xiwu Niu,[‡,‖] Sen Liu,[‖] and Guixing Ren*,[†]

[†]Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing 100081, People's Republic of China, [‡]Agronomy College, Shanxi Agricultural University, Taigu 030801, People's Republic of China, [§]Research Center for Environmental Science and Engineering, Shanxi University, Taiyuan 030006, People's Republic of China, and [‖]Shanxi Academy of Agricultural Sciences, Taiyuan 030031, People's Republic of China

The present study was to characterize the avenanthramide-rich extract (ARE) from oat bran and assess its effect on activity and gene expression of antioxidant enzymes in D-galactose-induced oxidative-stressed mice. High-performance liquid chromatography (HPLC) analysis found that ARE had 6.07% *N*-(3′,4′-dihydroxycinnamoyl)-5-hydroxyanthranilic acid (Bc), 4.37% *N*-(4′-hydroxycinnamoyl)-5-hydroxyanthranilic acid (Bp), and 5.36% *N*-(4′-hydroxy-3′-methoxycinnamoyl)-5-hydroxyanthranilic acid (Bf). In addition, ARE was also rich in vanillic acid (0.60%), caffeic acid (0.50%), syringic acid (0.54%), *p*-coumaric acid (0.16%), ferulic acid (0.08%), and sinapic acid (0.03%). Administration of D-galactose markedly lowered not only the activity of superoxide dismutase (SOD) and glutathione peroxidase (GPx) but also the gene expression of manganese superoxide dismutase (SOD), copper−zinc SOD, glutathione peroxidase (GPx), and lipoprotein lipase (LPL) mRNA in mice. Administration of ARE significantly reversed the D-galactose-induced oxidative stress by increasing the activity of the antioxidant enzymes and upregulating their gene expression. This was accompanied by a significant decrease in the malondialdehyde (MDA) level in mice given ARE compared to the control. The results demonstrated that ARE possessed the antioxidant activity and was effective against D-galactose-induced oxidative stress.

KEYWORDS: Avenanthramides; D-galactose; oxidative stress; mice

INTRODUCTION

D-Galactose has been used to induce the oxidative stress *in vivo* to mimic the natural aging in mice (*1−3*). D-Galactose is normally metabolized by galactose-1-phosphate uridyltransferase and D-galactokinase. When it is given in a large dose, D-galactose will be converted to galactitol, causing an osmotic stress with the production of reactive oxygen species (ROS) (*4*). In addition, D-galactose is a reducing monosaccharide and reacts with free amines to form the advanced glycation end product (AGEP), causing the activation of the advanced glycation end product receptor (RAGEP). When AGEP binds to RAGEP, the production of ROS occurs as well as cellular damage (*5*, *6*).

Oxidative damage may play a key role in the aging process (*7*). Aging and its related diseases may be a combination of free-radical-mediated oxidative damage and a weak endogenous antioxidant defense system (*8*). As one of the antioxidant defense systems, a group of enzymes, including superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase, are responsible for the removal of ROS, which may cause the changes of some biomarkers (*9*). Malondialdehyde (MDA) is a major biomarker that appears during the final stages of lipid peroxidation initiated by excessive ROS (*10*, *11*). An increase in the hepatic MDA concentration suggests the occurrence of lipid peroxidation, tissue damage, and failure of the antioxidant defense system to prevent the formation of excessive free radicals (*12*).

Oat (*Avena sativa* L.) is a commonly consumed whole-grain cereal and contains many kinds of phytochemicals that possess antioxidant properties, such as tocotrienols, phenolic acids, flavonoids, sterols, and phytic acid (*13*, *14*). Avenanthramides (AVEs), a group of alkaloids in oat that contain a phenolic group, have been reported to exhibit strong antioxidant activity both *in vitro* and *in vivo* (*15−18*). However, no study to date has addressed the effect of AVEs on the expression of antioxidative enzymes *in vivo*. The present study was therefore to investigate the effect of the oat avenanthramide-rich extract (ARE) on the activity and gene expression of antioxidant enzymes in D-galactose-induced oxidative-stressed mice.

MATERIALS AND METHODS

Chemicals. Commercial antioxidant assay kits for measuring SOD, GPx, and MDA were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Vanillic acid, caffeic acid, syringic acid, ferulic acid, sinapic acid, *p*-coumaric acid, and *trans*-cinnamic acid were purchased

*To whom correspondence should be addressed: Institute of Crop Science, Chinese Academy of Agricultural Sciences, Number 80 South Xueyuan Road, Haidian District, Beijing 100081, People's Republic of China. Telephone: +86-10-6211-5596. Fax: +86-10-6215-6596. E-mail: renguixing@caas.net.cn.

Published on Web 12/09/2010
© 2010 American Chemical Society

from Sigma-Aldrich (Sigma-Aldrich Corporation, St. Louis, MO). Three AVEs, namely, *N*-(3′,4′-dihydroxycinnamoyl)-5-hydroxyanthranilic acid (Bc), *N*-(4′-hydroxycinnamoyl)-5-hydroxyanthranilic acid (Bp), and *N*-(4′-hydroxy-3′- methoxycinnamoyl)-5-hydroxyanthranilic acid (Bf), were a gift from Dr. Mitchell L. Wise [Crop Research Unit, United States Department of Agriculture (USDA)] and Professor Atsushi Ishihara (Kyoto University, Kyoto, Japan) (**Figure 1**). The purity of all chemicals was of either analytical or chromatographic grade.

ARE Preparation. ARE was prepared from naked oats provided by the Chinese Academy of Agricultural Science (Beijing, China). The dry and cleaned oats were processed in a mill (Landert-Motoren AG, Buelach, Switzerland) to obtain the oat bran fraction. The oat bran sample was extracted with ethanol/water/acetic acid (80:20:0.1, v/v/v) in a solid−liquid ratio of 1:8 for 2 h at 40 °C and then centrifuged at 1250*g* for 10 min. The supernatant was filtered, and the solid residues were further extracted twice at 40 °C with the same solvent. All of the three extracted supernatants were pooled, concentrated, and then loaded onto an AB-8 resin. The AB-8 resin was washed using 0.1% acetic acid, and subsequently, the absorbed AVEs were eluted off the column using 95% ethanol. The ethanol fraction was sprayed, and the resultant ARE powder was stored at −20 °C.

Chemical Characterization of ARE. Determination of AVEs in ARE was performed on an Agilent 1200 series rapid resolution LC (Agilent Technologies, Santa Clara, CA) equipped with a ProSphere C18 analytical column (250 × 4.6 mm, 5 μm). Mobile phase A was 10 mM formic acid solution, whereas mobile phase B was acetonitrile (*19*). The gradient elution was programmed as follows: 10−20% B for 10 min, 20−60% B for 30 min, 60−20% B for 5 min, and 20−10% B for 5 min. The individual AVEs were monitored in an ultraviolet (UV) detector at 340 nm. Identification and quantification of Bc, Bp, and Bf in ARE were performed by comparing the retention times of authentic standards and areas of the external standard (**Figure 2**).

The phenolic compounds in the ARE were analyzed in a Shimadzu Prominence LC-20A (Shimadzu Corporation, Kyoto, Japan) equipped with an Alltima C18 column (250 × 4.6 mm, 5 μm, W. R. Grace and Co.-Conn., Columbia, MD). Mobile phase A consisted of a water solution containing 0.05% trifluoroacetic acid, while mobile phase B is a mixture of 30% acetonitrile, 10% methanol, 59.95% water, and 0.05% trifluoroacetic acid. A gradient program at a flow rate of 1.0 mL/min was as follows: 10−15% B for 15 min, 15−35% B for 5 min, 35−50% B for 15 min, 50−75% B for 10 min, and 75−10% B for 10 min. A UV detector at 280 nm was used to quantify phenolic compounds, namely, vanillic acid, caffeic acid, syringic acid, coumaric acid, ferulic acid, and sinapic acid in ARE.

Mice and D-Galactose Administration. Male Kun-Ming mice, at 1 week of age, were obtained from the Department of Laboratory Animal Science, Peking University Health Science Center (Beijing, China). Mice were kept under the standard conditions in an animal room with a 12 h light/dark cycle (light 7:00−19:00) at a temperature of 22 ± 2 °C and humidity of 60 ± 5%. The mice were allowed free access to food and water for 1 week prior to use. The diet was prepared according to the general quality standard for formula feeds of laboratory animals in China (GB14924.1). The whole experiment was carried out complying with the European Community guidelines for the use of experimental animals and approved by the Peking University Committee on Animal Care and Use.

A total of 72 mice were randomized into 6 groups (*n* = 12 each), namely, the normal control group, model group, three ARE groups, and vitamin E (VE) group. The mice in the model control, ARE, and VE groups were injected subcutaneously with D-galactose at the dose of 50 mg (kg of body weight)$^{-1}$ day^{-1}, while those of the normal control group were injected with the same volume of normal saline for 8 weeks. From the seventh week, the three experimental groups were given ARE at 250, 500, and 1000 mg (kg body weight)$^{-1}$ day^{-1} by intragastric gavage for 2 weeks after injection of D-galactose and the VE group mice received VE at 50 mg (kg body weight)$^{-1}$ day^{-1} by intragastric gavage for 2 weeks after injection of D-galactose. The model control group mice were administered the same volume of distilled water without ARE or VE. At the end of the experimental period, mice were sacrificed and the liver was collected and stored at −80 °C until analysis. Eight of the samples in each group were chosen for the subsequent assay.

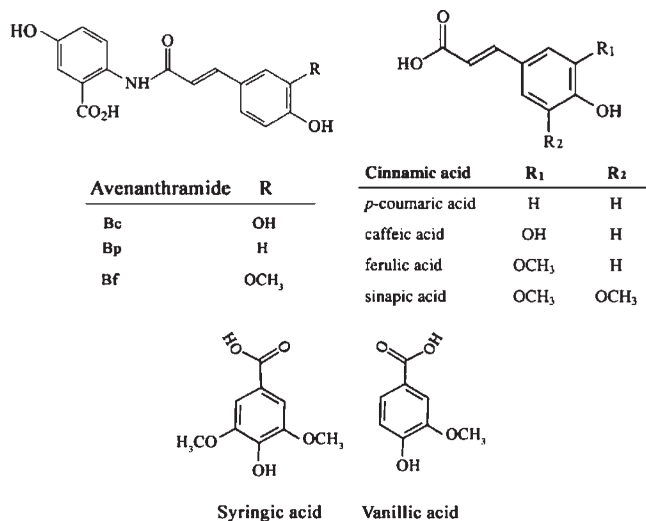

Figure 1. Structures of AVEs and phenolic compounds.

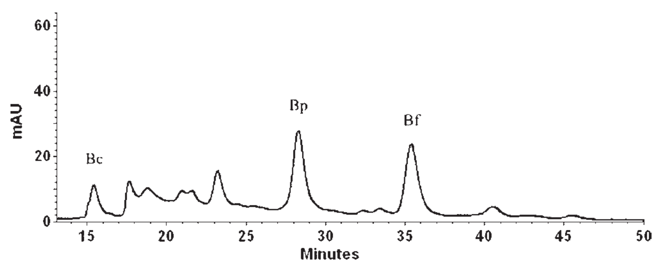

Figure 2. HPLC chromatogram of AVEs in oat ARE. Identification of peaks: Bc, *N*-(3′,4′-dihydroxycinnamoyl)-5-hydroxyanthranilic acid; Bp, *N*-(4′-hydroxycinnamoyl)-5-hydroxyanthranilic acid; Bf, *N*-(4′-hydroxy-3′-methoxycinnamoyl)-5-hydroxyanthranilic acid.

Tissue Preparation. After sacrifice, the liver was quickly lavaged in 15 mL of balanced Mg^{2+}/Ca^{2+}-free salt solution, then frozen in liquid nitrogen, and saved at −80 °C. The liver tissue was minced in an ice-cold solution containing 0.25 M sucrose, 10 mM Tris base, and 0.5 mM ethylenediaminetetraacetic acid (pH 7.4, 1:9, w/v) and then homogenized on ice in brief bursts by a Polytron homogenizer. The homogenate was centrifuged at 5000*g* (4 °C) for 10 min. The supernatant was used for various assays.

Activity of Antioxidant Enzymes. Total SOD and GPx in liver were measured by kits purchased from Nanjing Jiancheng Bioengineering Institute (NJBI, Nanjing, China). The protein content was measured by using the bicinchoninic acid protein assay kit (Sigma-Aldrich Corporation, St. Louis, MO) with bovine serum albumin as a standard. All measurements were performed in triplicates.

Lipid Peroxidation Level. Peroxidative damage to cellular lipid constituents was evaluated on the basis of the change of MDA content (*20*), which was measured in duplicates using the kits from NJBI.

Gene Expression of Antioxidant Enzymes. Total RNA was isolated from 100 mg of liver tissue using the Trizol reagent (Tiangen Biotech Co., Ltd., Beijing, China). RNA was resuspended in 40 μL of RNase-free water. The quantity and purity of the total RNA were analyzed spectrophotometrically. The primers used for examining the mRNA expression of each antioxidant enzyme were designed as in **Table 1**.

Real-time reverse transcription polymerase chain reaction (RT-PCR) was carried out in the iCycler iQ (Bio-Rad Laboratories, Inc., Hercules, CA). In brief, a master mix of the reaction components was prepared by mixing the following solutions: 6.5 μL of RNase-free water, 2 μL of forward primer (0.5 μm), 2 μL of reverse primer (0.5 μm), and 12.5 μL of Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA). A total of 40 μL of the Master Mix was then filled in each well of a 96-well microplate, and 2 μL of the sample containing 1 ng of total RNA was added as a PCR template. The RNA standards were also added to give

Table 1. Primers for Each Antioxidant Enzyme mRNA Expression

enzyme	forward primer	reverse primer
GAPDH	5′-GGTTGTCTCCTGCGACTTCA	3′-CCTCATTCTTTGGGACCTGGT
Mn SOD	5′-TGGGCAATGTGACTGCTGGA	3′-CGGACACCTCACTAACCCTAA
Cu−Zn SOD	5′-CTGGACAAACCTGAGCCCTAA	3′-GCACAGACACCCTCAGGTTC
GPx	5′-CCAACACCCAGTGACGACC	3′-CTTGGACTGTATCTTTGGGACG
LPL	5′-GAGTTTGGCTCCAGAGTTTGAC	3′-AGATTGACGGTGAAGTTGGTGT

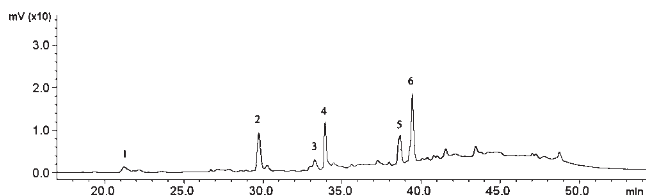

Figure 3. HPLC chromatogram of phenolic compounds in oat ARE. Identification of peaks: 1, vanillic acid; 2, caffeic acid; 3, syringic acid; 4, *p*-coumaric acid; 5, ferulic acid; and 6, sinapic acid.

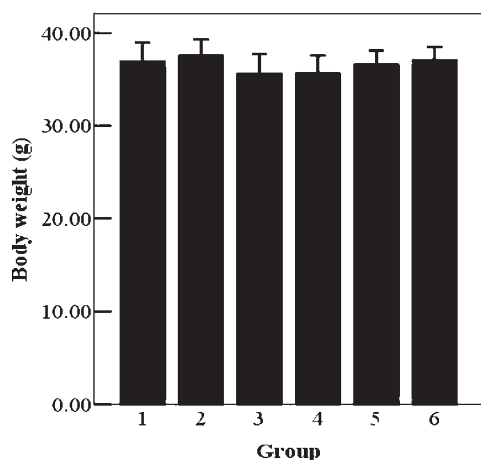

Figure 4. Effect of ARE on the body weight: group 1, normal control; group 2, model control; group 3, 250 mg of ARE/kg; group 4, 500 mg of ARE/kg; group 5, 1000 mg of ARE/kg; and group 6, 50 mg of VE/kg. Values are expressed as mean ± SD; $n = 8$ mice.

final concentrations of 0.04−25 ng of total RNA/RT-PCR reaction mixture. The following real-time RT-PCR protocol was used: reverse transcription, 42 °C for 1 h, 99 °C for 5 min, and 5 °C for 2 min; PCR initial activation, 95 °C for 15 min; amplification and quantification, 50 cycles of 95 °C for 20 s, 58 °C for 20 s, 72 °C for 35 s, and 75 °C for 15 s with fluorescence measurements; and melting curve analysis, 55−95 °C, with a heating rate of 0.5 °C/10 s and continuous fluorescent data from series dilution of total RNA. All samples were run in triplicates. The quantities of Mn SOD, Cu−Zn SOD, GPx, and lipoprotein lipase (LPL) in each sample were normalized to the corresponding total RNA based on the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) quantity and then expressed as the ratio of Mn SOD, Cu−Zn SOD, GPx, and LPL mRNA to GAPDH mRNA. The cycle number, at which a significant increase in the fluorescence signal was first detected, was defined as the threshold cycle. Using serial dilutions of the total RNA, a standard curve was generated on the basis of the linear relationship between the threshold cycle and the logarithm of the starting total RNA concentration. The melting curve analysis demonstrated that each of the primer pairs amplified a single predominant product with a distinct T_m, which was used to identify specific products in the subsequent analysis. As an additional control of specificity, the length of the PCR products was confirmed by agarose gel electrophoresis.

Statistical Analysis. Statistical analysis was performed using SPSS software package, version 13.0. The values were analyzed by one-way analysis of variance (ANOVA), followed by Duncan's multiple range test (DMRT). All of the results were expressed as mean ± standard deviation (SD). Differences of $p < 0.05$ were considered statistically significant. The gene expression data were analyzed using the $2^{-\Delta\Delta C_T}$ method (*21*).

Table 2. Effects of ARE on the Activities of Antioxidant Enzymes[a]

group	SOD (U/mg of protein)	GPx (U/mg of protein)
normal control	385.03 ± 49.50	485.24 ± 52.58
model control	244.62 ± 39.75[b]	370.75 ± 40.78[b]
ARE (250 mg/kg)	281.86 ± 38.04	402.33 ± 39.89
ARE (500 mg/kg)	337.03 ± 40.05[c]	431.04 ± 76.38[d]
ARE (1000 mg/kg)	379.06 ± 39.42[c]	477.79 ± 44.19[c]
VE (50 mg/kg)	369.08 ± 40.78[c]	465.20 ± 57.09[c]

[a] Values are expressed as mean ± SD for eight mice. [b] $p < 0.01$, compared to the normal control. [c] $p < 0.01$, compared to the model control. [d] $p < 0.05$, compared to the model control.

RESULTS

High-Performance Liquid Chromatography (HPLC) Analysis. The three major AVEs, Bc, Bp, and Bf, accounted for 60.72, 43.66, and 53.63 mg/g, respectively (**Figure 2**). Phenolic compounds were as follows: vanillic acid (6.01 mg/g), caffeic acid (4.98 mg/g), syringic acid (5.43 mg/g), *p*-coumaric acid (1.64 mg/g), ferulic acid (0.77 mg/g), and sinapic acid (0.31 mg/g) (**Figure 3**).

Body Weight. There was no significant difference in the body weight between the normal and model groups (**Figure 4**). Although the body weight in the ARE-treated group at the doses of 250 and 500 mg/kg was lesser than that in the model control group, the difference was not significant ($p > 0.05$).

Activity of Hepatic Antioxidant Enzymes. The model control mice had total SOD and GPx activities significantly decreased in comparison to the normal control group ($p < 0.01$; **Table 2**). ARE administration significantly increased the total SOD activity in a dose-dependent manner compared to the model group ($p < 0.01$). Similarly, ARE was also able to increase the GPx activity in a dose-dependent manner compared to the model control ($p < 0.05$ or $p < 0.01$). Upregulation of SOD and GPx activities by ARE at a dose of 1000 mg/kg of body weight was comparable to that observed for the VE-fed group.

Lipid Peroxidation. The model control had a significant increase in the hepatic MDA concentration compared to the normal group (**Figure 5**), confirming that D-galactose could induce the lipid oxidation. Administration of ARE at the doses of 500 or 1000 mg/kg could significantly reverse the rise in hepatic MDA caused by D-galactose ($p < 0.01$). Similarly, the VE group could have the hepatic MDA level reduced in comparison to the model control ($p < 0.01$).

mRNA Expression of Mn SOD, Cu−Zn SOD, GPx, and LPL. D-Galactose led to a reduction of Mn SOD, Cu−Zn SOD, GPx, and LPL mRNA expression (**Figure 6**). However, administration of ARE at the doses of 500 and 1000 mg/kg upregulated the expression of mRNA compared to those in the model group ($p < 0.05$ or $p < 0.01$). The similar upregulation was also seen in the VE-treated group compared to the model control ($p < 0.01$).

DISSCUSSION

It has been shown that D-galactose exposure can induce the oxidative damage *in vivo* (*22*, *23*). The present study clearly demonstrated that administration of D-galactose caused a severe oxidative stress accompanied with various aging symptoms, while supplementation of ARE was able to partially reverse these adverse effects. Similar results were shown in previous studies. An AVE-enriched mixture (1 g/kg) extracted from oat could enhance some antioxidant defenses *in vivo* (*24*). Even oat supplementation (10 g/kg) could also attenuate alcohol-induced oxidative stress by inhibiting NO overproduction (*25*).

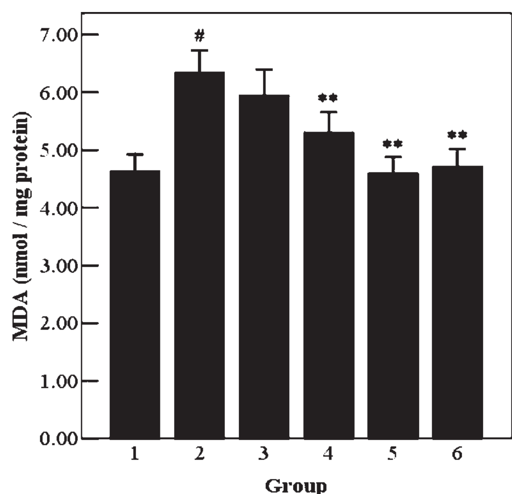

Figure 5. Effect of ARE on hepatic MDA: group 1, normal control; group 2, model control; group 3, 250 mg of ARE/kg; group 4, 500 mg of ARE/kg; group 5, 1000 mg of ARE/kg; and group 6, 50 mg of VE/kg. Values are expressed as mean ± SD; $n = 8$ mice. (#) $p < 0.05$, compared to the normal group. (**) $p < 0.01$, compared to the model control group.

SOD serves as a first gatekeeper in the antioxidant defense system to scavenge superoxide anion (*26*), while GPx is the catalyzer, which activates the reaction of lipid hydroperoxides with reduced glutathione to form glutathione disulfide (*27*). An AVE-enriched mixture (1 g/kg) extracted from oat was reported to increase human plasma GPx activity by 30−35% (*24*). In this study, SOD and GPx were significantly downregulated in D-galactose-treated groups, whereas ARE supplementation restored the activity and upregulated these two enzymes. A beneficial effect associated with supplementation of ARE at a dose of 1000 mg/kg was comparable to that with supplementation of 50 mg/kg of VE. Because there was no significant difference between 1000 mg/kg of ARE and 50 mg/kg of VE, we could infer that the antioxidant activities of ARE were weaker than those of VE on the same weight basis. The results suggested that ARE possessed the effective antioxidants and was able to prevent the D-galactose-induced oxidative stress.

Lipid peroxidation occurs when ROS attack the membrane polyunsaturated fatty acids, leading to their disintegration and

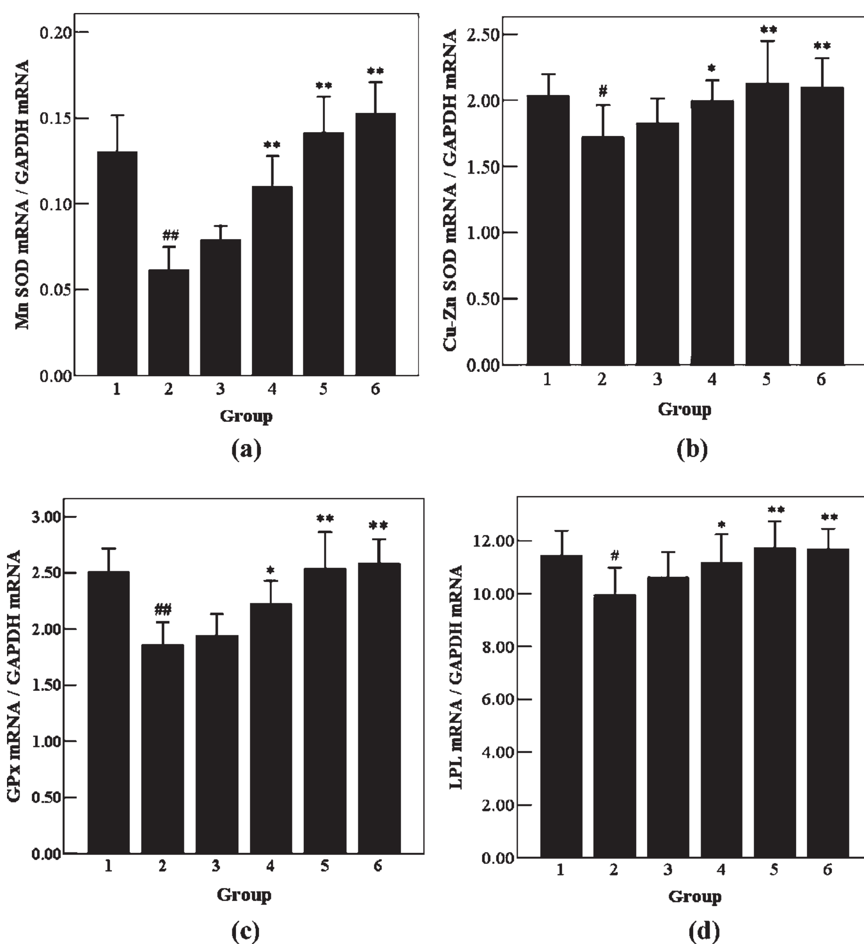

Figure 6. Effects of ARE on the expression of hepatic (a) Mn SOD, (b) Cu−Zn SOD, (c) GPx, and (d) LPL mRNA: group 1, normal control; group 2, model control; group 3, 250 mg of ARE/kg; group 4, 500 mg of ARE/kg; group 5, 1000 mg of ARE/kg; and group 6, 50 mg of VE/kg. The expression level for each gene was determined using real-time RT-PCR and expressed as the relative mRNA level (per GAPDH mRNA). Values are expressed as mean ± SD; $n = 8$ mice. (#) $p < 0.05$ and (##) $p < 0.01$, compared to the normal group. (*) $p < 0.05$ and (**) $p < 0.01$, compared to the model control.

formation of short-chain alkyl radicals and aldehydes (*22*). MDA is a major reactive aldehyde that is formed during the final stages of lipid peroxidation of biological membrane polyunsaturated fatty acid (*11*). An increase in the hepatic MDA concentration suggests enhanced peroxidation, leading to tissue damage and failure of the antioxidant defense system to prevent the formation of excessive free radicals (*12*). Our results demonstrated that administration of D-galactose caused an increase in the hepatic MDA concentration, while supplementation of ARE decreased the level of MDA, especially at the doses of 500 and 1000 mg/kg, suggesting that ARE was effective in scavenging the free radicals and suppressing the production of MDA.

Natural phenolic acids have been reported to induce selectively hepatic mRNA transcripts for Cu−Zn SOD, GPx, and catalase and, therefore, upregulate the gene expression of these antioxidant enzymes (*28*). It has been shown that the basic region leucine-zipper factor, nuclear-factor-E2-related factor (Nrf2), is essential for antioxidant responsive element-mediated induction (*29*, *30*). Antioxidant enzyme mRNA expression has been evaluated previously using real-time RT-PCR (*31*−*35*). However, there has been no report to date about antioxidant enzyme mRNA expression in D-galactose-induced aging mice. The present study found that supplementation of ARE enhanced the expression of Mn SOD, Cu−Zn SOD, and GPx mRNA possibly because of the upregulation of gene transcription as well as the Nrf2 transcription factor. In addition, LPL, a key enzyme that regulates lipoprotein metabolism, might further regulate cellular lipid levels. Reduction of LPL in the body may lead to an increased blood triglyceride level and a decreased high-density lipoprotein cholesterol level and, thus, increase the risk of atherosclerosis (*36*). The present results demonstrated that ARE significantly increased LPL mRNA expression in aging mice, indicating that ARE could potentially prevent atherosclerosis.

In summary, the present study found that ARE was rich in AVEs and phenolic antioxidants. Administration of D-galactose caused the oxidative stress, decreased the antioxidant enzyme activity, and increased the MDA level. Supplementation of ARE could restore the antioxidant defense system by increasing the activity and upregulating the gene expression of antioxidant enzymes. Therefore, AREs will have the potential to be further explored as an antioxidant functional food in the prevention of aging-related diseases.

ABBREVIATIONS USED

AGEP, advanced glycation end product; ARE, avenanthramide-rich extract; AVE, avenanthramide; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GPx, glutathione peroxidase; LPL, lipoprotein lipase; MDA, malondialdehyde; RAGEP, receptor for advanced glycation end product; RT-PCR, reverse transcription polymerase chain reaction; SOD, superoxide dismutase; VE, vitamin E.

ACKNOWLEDGMENT

We thank Dr. Mitchell L. Wise and Professor Atsushi Ishihara for providing pure AVE standards. We also thank Professor Jianmin Wan, Director of Institute of Crop Science, Chinese Academy of Agricultural Science, for allowing us to use his facilities.

LITERATURE CITED

(1) Ho, S. C.; Liu, J. H.; Wu, R. Y. Establishment of the mimetic aging effect in mice caused by D-galactose. *Biogerontology* **2003**, *4*, 15−18.

(2) Song, X.; Bao, M.; Li, D.; Li, Y. M. Advanced glycation in D-galactose induced mouse aging model. *Mech. Ageing Dev.* **1999**, *108*, 239−251.

(3) Lei, M.; Hua, X. D.; Xiao, M.; Ding, J.; Han, Q. Y.; Hu, G. Impairments of astrocytes are involved in the D-galactose-induced brain aging. *Biochem. Biophys. Res. Commun.* **2008**, *369*, 1082−1087.

(4) Hsieh, H. M.; Wu, W. M.; Hu, M. L. Soy isoflavones attenuate oxidative stress and improve parameters related to aging and Alzheimer's disease in C57BL/6J mice treated with D-galactose. *Food Chem. Toxicol.* **2009**, *47*, 625−632.

(5) Karasek, M. Melatonin, human aging, and age-related diseases. *Exp. Gerontol.* **2004**, *39*, 1723−1729.

(6) Baynes, J. W. The role of AGEs in aging: Causation or correlation. *Exp. Gerontol.* **2001**, *36*, 1527−1537.

(7) Floyd, R. A.; Hensley, K. Oxidative stress in brain aging. Implications for therapeutics of neurodegenerative diseases. *Neurobiol. Aging* **2002**, *23*, 795−807.

(8) Jang, Y. C.; Remmen, V. H. The mitochondrial theory of aging: Insight from transgenic and knockout mouse models. *Exp. Gerontol.* **2009**, *44*, 256−260.

(9) Sreelatha, S.; Padma, P. R.; Umadevi, M. Protective effects of *Coriandrum sativum* extracts on carbon tetrachloride-induced hepatotoxicity in rats. *Food Chem. Toxicol.* **2009**, *47*, 702−708.

(10) Nordmann, R.; Ribière, C.; Rouach, H. Implication of free radical mechanisms in ethanol-induced cellular injury. *Free Radical Biol. Med.* **1992**, *12*, 219−240.

(11) Vaca, C. E.; Wilhelm, J.; Harms-Ringdahl, M. Interaction of lipid peroxidation products with DNA. A review. *Mutat. Res.* **1988**, *195*, 137−149.

(12) Naik, S. R. Antioxidants and their role in biological functions: An overview. *Indian Drugs* **2003**, *40*, 501−516.

(13) Collins, F. W.; McLachlan, D. C.; Blackwell, B. A. Oat phenolics: Avenalumic acids, a new group of bound phenolic acids from oat groats and hulls. *Cereal Chem.* **1991**, *68*, 184−189.

(14) Peterson, D. M. Oat antioxidants. *J. Cereal Sci.* **2001**, *33*, 115−129.

(15) Bryngelsson, S.; Dimberg, L. H.; Kamal-Eldin, A. Effects of commercial processing on levels of antioxidants in oats (*Avena sativa* L.). *J. Agric. Food Chem.* **2002**, *50*, 1890−1896.

(16) Lee-Manion, A. M.; Price, R. K.; Strain, J. J.; Dimberg, L. H.; Sunnerheim, K.; Welch, R. W. *In vitro* antioxidant activity and antigenotoxic effects of avenanthramides and related compounds. *J. Agric. Food Chem.* **2009**, *57*, 10619−10624.

(17) Dimgerg, L. H.; Theander, O.; Lingnert, H. Avenanthramides—A group of phenolic antioxidants in oats. *Cereal Chem.* **1993**, *70*, 637−641.

(18) Ji, L. L.; Lay, D.; Chung, E.; Ying, F.; Peterson, D. M. Effects of avenanthramides on oxidant generation and antioxidant enzyme activity in exercised rats. *Nutr. Res.* (*N. Y., NY, U. S.*) **2003**, *23*, 1579−1590.

(19) Jastrebova, J.; Skoglund, M.; Nilsson, J.; Dimberg, L. H. Selective and sensitive LC−MS determination of avenanthramides in oats. *Chromatographia* **2006**, *63*, 419−423.

(20) Mihara, M.; Uchiyama, M. Determination of malondialdehyde precursor in tissues by thiobarbituric acid test. *Anal. Biochem.* **1978**, *86*, 271−278.

(21) Livak, K. J.; Schmittgen, T. D. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C_T}$ method. *Methods* **2001**, *25*, 402−408.

(22) Cui, X.; Zuo, P.; Zhang, Q.; Li, X.; Hu, Y.; Long, J.; Packer, L.; Liu, J. Chronic systemic D-galactose exposure induces memory loss, neurodegeneration, and oxidative damage in mice: protective effects of *R*-α-lipoic acid. *J. Neurosci. Res.* **2006**, *84*, 647−654.

(23) Wei, H.; Li, L.; Song, Q.; Ai, H.; Chu, J.; Li, W. Behavioural study of the D-galactose induced aging model in C57BL/6J mice. *Behav. Brain Res.* **2005**, *157*, 245−251.

(24) Chen, C. O.; Milbury, P. E.; Collins, F. W.; Blumberg, J. B. Avenanthramides are bioavailable and have antioxidant activity in humans after acute consumption of an enriched mixture from oats. *J. Nutr.* **2007**, *137*, 1375−1382.

(25) Tang, Y.; Forsyth, C. B.; Banan, A.; Fields, J. Z.; Keshavarzian, A. Oats supplementation prevents alcohol-induced gut leakiness in rats

by preventing alcohol-induced oxidative tissue damage. *J. Pharmacol. Exp. Ther*. **2009**, *329*, 952−958.
(26) Reiter, R. J.; Tan, D.; Osuna, C.; Gitto, E. Actions of melatonin in the reduction of oxidative stress. *J. Biomed. Sci.* **2000**, *7*, 444−458.
(27) Venukumar, M. R.; Latha, M. S. Antioxidant activity of *Curculigo orchioides* in carbon tetrachloride-induced hepatopathy in rats. *Indian J. Clin. Biochem*. **2002**, *17*, 80−87.
(28) Yeh, C. T.; Yen, G. C. Induction of hepatic antioxidant enzymes by phenolic acids in rats is accompanied by increased levels of multidrug resistance-associated protein 3 mRNA expression. *J. Nutr*. **2006**, *136*, 11−15.
(29) Kobayashi, M.; Yamamoto, M. Molecular mechanisms activating the Nrf2−Keap1 pathway of antioxidant gene regulation. *Antioxid. Redox Signaling* **2005**, *7*, 385−394.
(30) Keum, Y. S.; Owuor, E. D.; Kim, B. R.; Hu, R.; Kong, A. N. Involvement of Nrf2 and JNK1 in the activation of antioxidant responsive element (ARE) by chemopreventive agent phenethyl isothiocyanate (PEITC). *Pharm. Res*. **2003**, *20*, 1351−1356.
(31) Martin, R.; Fitzl, G.; Mozet, C.; Martin, H.; Welt, K.; Wieland, E. Effect of age and hypoxia/reoxygenation on mRNA expression of antioxidative enzymes in rat liver and kidneys. *Exp. Gerontol*. **2002**, *37*, 1481−1487.
(32) Liu, W.; Saint, D. A. Validation of a quantitative method for real time PCR kinetics. *Biochem. Biophys. Res. Commun*. **2002**, *294*, 347−353.
(33) Hatao, H.; Oh-ishi, S.; Itoh, M.; Leeuwenburgh, C.; Ohno, H.; Ookawara, T.; Kishi, K.; Yagyu, H.; Nakamura, H.; Matsuoka, T. Effects of acute exercise on lung antioxidant enzymes in young and old rats. *Mech. Ageing Dev*. **2006**, *127*, 384−390.
(34) Hollander, J.; Bejma, J.; Ookawara, T.; Ohno, H.; Ji, L. L. Superoxide dismutase gene expression in skeletal muscle: Fiber-specific effect of age. *Mech Ageing Dev*. **2000**, *116*, 33−45.
(35) Cao, L.; Leers-Sucheta, S.; Azhar, S. Aging alters the functional expression of enzymatic and non-enzymatic anti-oxidant defense systems in testicular rat Leydig cells. *J. Steroid Biochem. Mol. Biol*. **2004**, *88*, 61−67.
(36) Yagyu, H.; Chen, G.; Yokoyama, M.; Hirata, K.; Augustus, A.; Kako, Y.; Seo, T.; Hu, Y.; Lutz, E. P.; Merkel, M.; Bensadoun, A.; Homma, S.; Goldberg, I. J. Lipoprotein lipase (LpL) on the surface of cardiomyocytes increases lipid uptake and produces a cardiomyopathy. *J. Clin. Invest*. **2003**, *111*, 419−426.

Received for review October 9, 2010. Revised manuscript received November 23, 2010. Accepted November 23, 2010. This study was supported by the Technology Transformation Fund (2008GBZA300040) from the Ministry of Science and Technology, People's Republic of China, and an Earmarked Fund for the Modern Agro-industry Technology Research System (CYTX-014) from the Ministry of Agriculture, People's Republic of China.