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Chemical Characterization of a Procyanidin-Rich Extract from Sorghum Bran and Its Effect on Oxidative Stress and Tumor Inhibition in Vivo

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ABSTRACT: The present study was to characterize a procyanidin-rich extract (PARE) from sorghum (*Sorghum bicolor* (L.) Moench) bran and assess its biological activities. The procyanidin oligomers were separated and identified by normal-phase HPLC equipped with fluorescence (FLD) and mass spectrometry (MS) detectors. In addition, the effects of PARE on oxidative stress in mice induced by D-galactose as well as tumor inhibition in C57BL/6J mice bearing Lewis lung cancer were investigated. Administration of D-galactose significantly ($p < 0.05$) lowered the activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px). This was accompanied by a significant ($p < 0.05$) increase in malondialdehyde (MDA) levels in both liver and serum. Administration of PARE (150 mg/kg) significantly ($p < 0.05$) reversed the D-galactose-induced oxidative stress by enhancing the activities of antioxidant enzymes. Furthermore, PARE administration inhibited tumor growth and metastasis formation by suppressing vascular endothelial growth factor (VEGF) production. The results suggested that PARE had antioxidant and antitumor activities.

KEYWORDS: sorghum procyanidins, D-galactose, oxidative stress, VEGF, lung cancer, antitumor activity

INTRODUCTION

Sorghum contains various phytochemicals such as procyanidins, phenolic acids, anthocyanins, phytosterols, and policosanols[1,2] and is an important cereal crop both in China and worldwide. Procyanidins are oligomers and polymers of flavan-3-ol units. It was reported that procyanidins had effective antioxidant, antiobesity, antiallergy, antihypertensive, and antitumor activities.[3−6] Sorghum bran is concentrated with procyanidins, which may be potentially exploited for health in humans.[7]

Free radical damage has been implicated as a major contributor to cancer.[8,9] According to Harman's hypothesis, reactive oxygen species (ROS) produce cumulative damage of cellular macromolecules.[10] In such a case, immunity can be compromised; even worse, DNA codes can be altered. The damage to DNA caused by free radicals could promote the cause of cancer.[9]

There has been an increased interest in finding natural antioxidants from plants to scavenge free radicals. Although many extracts from plants have been proved to be effective in the treatment of oxidative damage and other age-associated diseases.[11] However, no study to date has addressed the effect of the procyanidin-rich extract (PARE) from sorghum bran against oxidative damage in mice.

Despite some research on procyanidins from many food sources, a systematic study of PARE from sorghum bran on the chemical characterization and its biological activities is lacking. The present study is intended to provide a scientific basis for the comprehensive utilization of sorghum bran and therefore was carried out to (i) characterize the procyanidin-rich extract (PARE) from sorghum bran by normal-phase HPLC-MS, (ii) investigate the effect of PARE on oxidative stress in mice, and (iii) determine the effect of PARE administration on lung tumor growth and vascular endothelial growth factor (VEGF) production in mice bearing Lewis lung cancer.

MATERIALS AND METHODS

Chemicals. Procyanidins standard (a mixture of oligomeric and polymeric procyanidin, HPLC grade, ≥95% pure) was purchased from JF-Natural (Tianjin, China). Commercial antioxidant assay kits for malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) were purchased from Nanjing Jiancheng Bioengineering Institute (NJBI; Nanjing, China). D-Galactose, gallic acid, and Folin−Ciocalteu's reagent were purchased from Sigma-Aldrich (Shanghai, China), and cyclophosphamide (CTX) was from Shanghai Richem International Co., Ltd. (Shanghai, China). VEGF antibody was purchased from Abcam (Hong Kong, China). Other reagents were of analytical or chromatography grade.

Sample Extraction and Purification. Sorghum grains [*Sorghum bicolor* (Linn.) Moench] were supplied by Shanxi Academy of Agricultural Sciences (Taiyuan, China). The dried and cleaned sorghum grains were processed in a mill (Landert-Motoren AG, Buelach, Switzerland) to obtain sorghum bran fraction. The powder passing through 0.25 mm

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Table 1. Composition of the Experimental Diets

content	Kunming mice	C57BL/6J mice
corn starch (%)	40.3	40.3
soybean meal (%)	16.6	16.6
soybean powder (%)	5.9	5.9
wheat flour (%)	20	20
wheat bran (%)	10.6	10.6
fish powder (%)	1.2	1.2
$CaCO_3$ (%)	1.9	1.9
$CaPO_4$ (%)	2.2	2.2
mineral (%)	0.8	0.8
vitamin (%)	0.1	0.1
soybean oil (%)	0.4	0.4

screen was extracted in 70% ethanol, solid−liquid ratio (1:10), for 1 h at 70 °C. After filtering, the solution was evaporated on a rotary evaporator. The extract was concentrated and then loaded onto an AB-8 resin. The absorbed procyanidins were eluted off column using 30% ethanol. The ethanol fraction was sprayed, and the resultant PARE powder was stored at −20 °C.

Chemical Characterization of PARE. The total phenolic content in PARE was spectrophotometrically determined according to the Folin−Ciocalteu procedure.[12] Additionally, the total content of procyanidins in PARE was measured by *n*-butanol/HCl/Fe method.[13] The procyanidin oligomers in PARE were analyzed according to the method of Hellstrom et al.[14] with slight modifications. The characterization and detection of PARE were performed on an Agilent 1200 liquid chromatograph (Agilent Technologies, Santa Clara, CA) system equipped with diode array and fluorescence detectors. Normal-phase HPLC with a 250 × 4.6 mm i.d., 5 μm, silica Luna column (Phenomenex Inc., Darmstadt, Germany) was used with the column temperature set at 35 °C. The injection volume was 10 μL. The mobile phase consisted of (A) dichloromethane/methanol/water/acetic acid (82:14:2:2) and (B) dichloromethane/methanol/water/acetic acid (10:86:2:2). The gradient was as follows: 0−35 min, 0−13.5% B, linear; 35−65 min, 13.5−29.2% B, linear; 65−70 min, 29.2−100% B, linear; 70−75 min, 100% B, linear, followed by 10 min of re-equilibration of the column before the next run. The flow rate was maintained at 1 mL/min. The extract was identified using both UV (λ = 280 nm), fluorescence detectors (FLD) (λ_{ex} = 280 nm, λ_{em} = 323 nm), and electrospray ionization tandem mass (ESI-MS/MS) spectrometry.

Treatments of D-Galactose-Treated Mice. Male Kun-Ming mice, aged 2.5 months, weighing 20 ± 2 g, 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 a.m.−7:00 p.m.) at a temperature of 22 ± 2 °C and a relative humidity of 60 ± 5%. The diet was prepared according to the general quality standard for formula feeds of laboratory animals in China (GB14924.1) (shown in Table 1). The whole experiment was carried out in compliance with European Community guidelines for the use of experimental animals and approved by the Peking University Committee on Animal Care and Use.

According to preliminary experiments and previous literature,[15−17] the mice were randomly divided into four groups (n = 10 each), namely, a control group, a model group, a PARE group, and a vitamin E (VE) group. Except for the control group, mice were subcutaneously injected with D-galactose at the dose of 50 mg/kg body weight (BW) once daily for 7 weeks, whereas those of the control group were treated with the same volume of physiological saline. For the PARE and VE groups, according to our previous study, mice were simultaneously subcutaneously injected with PARE at the dose of 150 mg/kg and with vitamin E at the dose of 50 mg/kg, respectively, once daily for 7 weeks. Control group and model group mice were administered the same volume of physiological saline. At the end of the experimental period, animals were sacrificed to collect livers and blood. After centrifugation, the serum was obtained. These were stored at −80 °C until analysis.

Tumor Cell Preparation. Lewis lung cancer cells were purchased from Cancer Institute and Hospital, Chinese Academy of Medical Sciences (Beijing, China). The cells were cultured in Eagle's minimal essential medium, supplemented with 10% fetal bovine serum (Hyclone, Logan, UT), 100 mg/mL penicillin, and 100 mg/mL streptomycin from Invitrogen (Carlsbad, CA) and maintained in an incubator with a humidified atmosphere of 5% CO_2 at 37 °C.

To generate source tumors, tumor cells were injected subcutaneously into the flanks of C57BL/6J mice.[18] When tumors grew to approximately 20 mm in diameter, they were removed, minced, and made into a suspension with the concentration of 2×10^7 cells/mL.

Treatment of Mice Bearing Lewis Lung Cancer. Fifty male mice (C57BL/6J) aged 4−6 weeks, weighing 18 ± 2 g, were purchased from the Department of Laboratory Animals in Peking University Third Hospital (Beijing, China). The same feeding conditions and composition of the diets fed to the mice as mentioned above were used. Cell suspension (0.2 mL) was subcutaneously inoculated into the right thighs of mice. All of the mice were randomly divided into five groups (n = 10 each), namely, a control group, a CTX group, and three PARE groups. After 24 h of tumor inoculation, the treatments were as follows: (1) control group mice were administered physiological saline alone (intraperitoneal injection, ip) for 17 consecutive days; (2) CTX group mice were treated with cyclophosphamide (CTX, 30 mg/kg BW, ip) once every other day; (3) mice of the PARE groups, namely, PARE low-dose (PAREL) group, PARE middle-dose (PAREM) group, and PARE high-dose (PAREH) group, were administered PARE orally at doses of 100, 200, and 400 mg/kg (BW), respectively, for 17 consecutive days. Beginning 4 days after inoculation, tumor diameters were measured every 2 days, and 18 days after tumor transplantation, mice were sacrificed for analysis.[19]

Biochemical Assays. SOD, GSH-Px in liver homogenates, and serum were determined by using commercially available NJBI kits. The contents of MDA in liver and serum were also determined by using NJBI kits. All procedures completely complied with the manufacturer's instructions.

Solid Tumor Growth and Metastasis Assays. The C57BL/6J mice bearing Lewis lung cancer were executed for determination of tumor size. The lungs were removed, and metastasis was counted according to the method described by Bing et al. with a slight modification.[20] The effects of the oral administration of PARE on tumor growth and metastasis formation were measured as follows: (1) tumor inhibition rate (%) = $(C - T)/C \times 100\%$ (T and C represent the average tumor weights of treatment group and control group, respectively); (2) tumor volume, $V = \pi/6 \times L \times W \times H$ (where L, W, and H are the three axes of tumors);[21] the tumor diameters were measured every 2 days with calipers and expressed as volume; and (3) number of metastasis on the lung epidermis.

Immunohistochemistry Assays. Immunohistochemistry was performed as previously described.[20,22] Paraffin-embedded tumor specimens were cut into 4 μm sections. Hematoxylin and eosin (HE) staining was used to examine potential differences in vascularity. Sections were deparaffinized in xylene and dehydrated in graded alcohols. After washing in phosphate-buffered saline (PBS), sections were placed in EDTA buffer antigen retrieval (Epicenter Biotechnologies, Beijing, China) at 95−97 °C for 20 min and then were subsequently cooled at room temperature for an additional 20 min. Sections were then blocked with 10% goat serum in PBS, followed by a block for endogenous peroxidases with 3% H_2O_2. Sections were incubated overnight at 4 °C

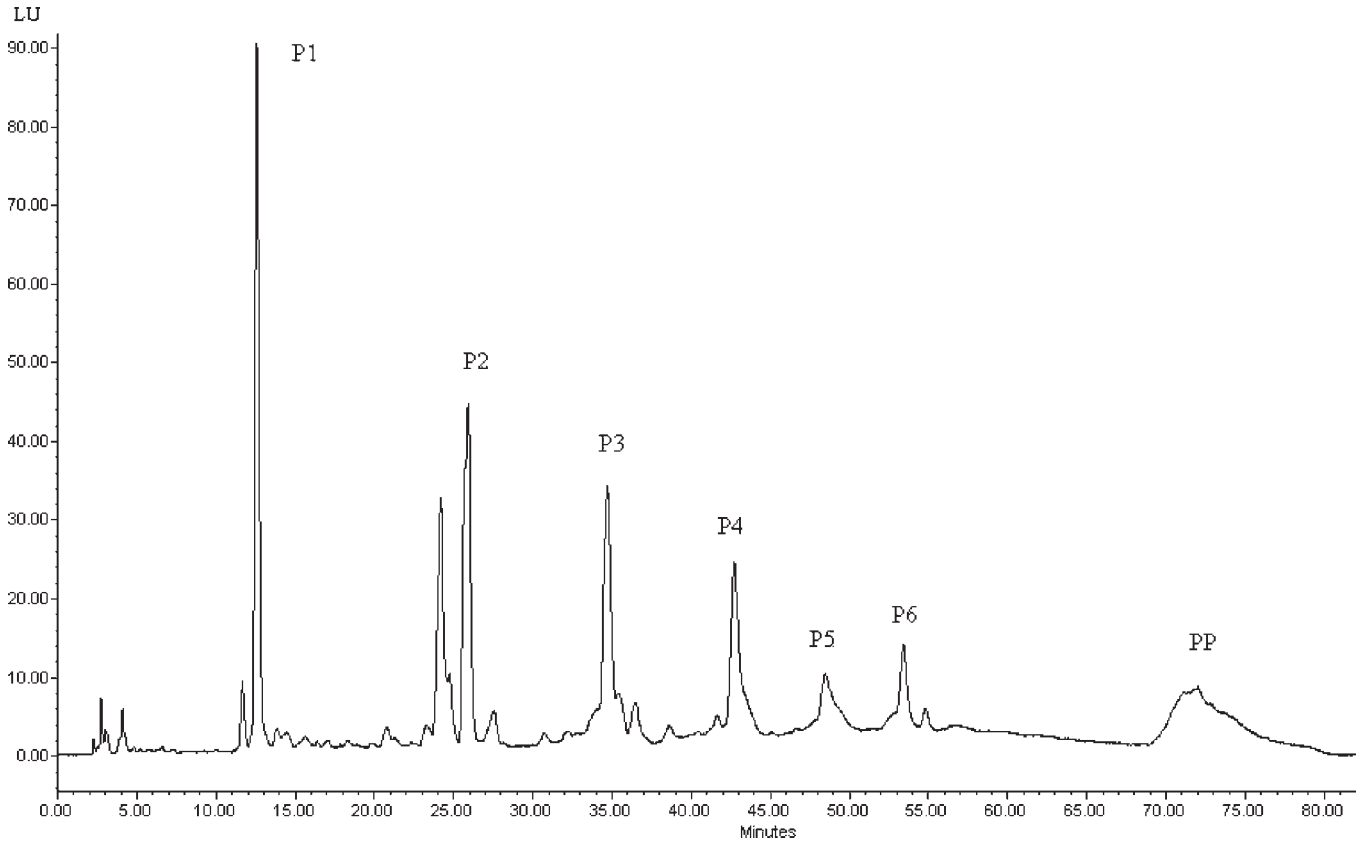

Figure 1. Normal-phase HPLC-FLD trace of proanthocyanidin-rich extract (PARE) from sorghum bran. Labels P1−P6 indicate the degrees of polymerization (DP) of procyanidins in the peaks. Polymeric proanthocyanidins (PP) appear as a single peak at the end of the chromatogram. LU, luminescence units.

with biotinylated rabbit anti-goat secondary antibody (Abcam Biotechnology, Hong Kong, China) followed by horseradish peroxidase (Sigma-Aldrich). Unbound antibodies were removed the following day by washing the slides three times with PBS. Areas positive for VEGF were stained brown after development with diaminobenzidine. Slides were counterstained with filtered Mayer's hematoxylin (Sigma-Aldrich), rinsed with distilled water, allowed to dry, and then mounted for viewing purposes. Five 200× bright-field images were taken from each section. The percentage in area of positively stained epidermis for VEGF was quantified using Image-Pro image analysis software (Media Cybernetics, version 7.0).

Statistical Analysis. Statistical analysis was performed using the SPSS software package, version 13.0. The values were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple-range test. All of the results were expressed as the mean ± SD in each group. Differences of $p < 0.05$ were considered to be statistically significant.

RESULTS AND DISCUSSION

Chemical Characterization Analysis. The contents of total phenolic and total procyanidins in PARE were 86.9 and 54.68%, respectively. The procyanidins were separated according to their degree of polymerization (DP) (Figure 1). The procedure allowed the precise separation of PARE from monomer (denoted P1) to hexamer (P6) (Table 2); these oligomers were identified by comparing their retention time and MS data with published data.[14,23,24] Polymeric procyanidins do not resolve by normal-phase HPLC, and so they were collected as a single peak (PP), as previously demonstrated by Gu et al.[23] Additionally, the contribution of the individual oligomers to the total procyanidins was calculated by the peak area measurement (Table 2).

Table 2. Individual Oligomers, Molecular Ions, Contribution (%) to the Total Procyanidins, and Retention Times (t_R) Determined in PARE

DP[a]	oligomers	contribution[b] (%)	molecular ion	t_R (min)
1	monomer	22.7	289	12.561
2	dimer	18.1	577	25.925
3	trimer	14.6	865	34.947
4	tetramer	11.0	1153	43.121
5	pentamer	4.4	1441	48.596
6	hexamer	3.3	1729	53.590
P[c]	ND[d]	8.0	ND	71.982

[a] Degree of polymerization. [b] The contribution of the individual oligomers to the total procyanidins was calculated by the peak area measurement. [c] Mixture of polymers, as a single peak. [d] ND, not detected.

In this study, procyanidin oligomers from monomers to hexamers were separated and identified; however, for higher oligomers, the molecular ions could not be detected due to the limited scanning range of the mass spectrometer used. Thus, future work is needed to study the chemical characteristics of higher procyanidin oligomers and polymers.

Effect of PARE on MDA Levels in Liver and Serum. It has been shown that D-galactose exposure can induce oxidative stress

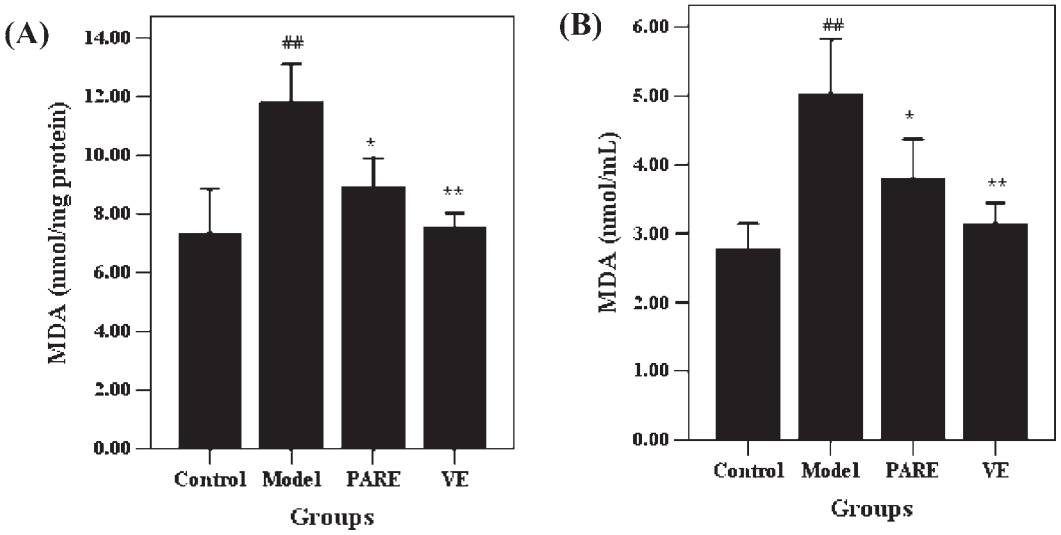

Figure 2. Effect of PARE from sorghum bran on MDA levels: (A) MDA concentration in liver; (B) MDA concentration in serum. Values are expressed as the mean ± SD, $n = 10$ mice. (##) $p < 0.01$, compared with the control group; (*) $p < 0.05$ and (**) $p < 0.01$, compared with the model group.

Table 3. Effects of PARE from Sorghum Bran on D-Galactose-Induced Aging Mice[a]

	SOD		GSH-Px	
group	serum (NU/mL)	hepatic (U/mg protein)	serum (NU/mL)	hepatic (U/mg protein)
control	118.2 ± 4.15	63.79 ± 6.05	181.22 ± 8.67	482.96 ± 43.1
model	99.54 ± 5.83 ##	50.92 ± 5.34 ##	115.52 ± 6.94 ##	368.05 ± 24.27 ##
VE	110.32 ± 6.98 **	60.76 ± 5.16 **	165.49 ± 5.64 **	466.64 ± 41.73 **
PARE	105.63 ± 7.70 *+	57.48 ± 4.36 *	151.14 ± 40.61 *+	436.93 ± 36.91 *+

[a] Values are expressed as the mean ± SD. (##) $p < 0.01$, compared with the control group; (*) $p < 0.05$ and (**) $p < 0.01$, compared with the model group; (+) $p < 0.05$, VE group compared with PARE group, $n = 10$.

by increasing lipid peroxidation and decreasing antioxidant enzyme activities in vivo.[15,16] MDA is a major reactive aldehyde that appears during the peroxidation of biological membrane polyunsaturated fatty acid and is widely used as a biomarker of oxidative stress.[25] Therefore, the MDA concentration is used as an indicator of tissues involving oxidative damage.[26] The levels of MDA both in liver and in serum of mice are shown in Figure 2. The model control had a significant ($p < 0.01$) increase in MDA concentration compared to the normal group both in liver and in serum, confirming that D-galactose could induce lipid oxidation.

In the present study, administration of PARE at the dose of 150 mg/kg significantly ($p < 0.05$) reversed the rise in MDA levels in both liver homogenate and serum, whereas the vitamin E group (50 mg/kg BW) had significantly reduced MDA levels ($p < 0.01$) in both liver and serum compared with the model control group. This was in accordance with the previous paper from our laboratory, which showed that mice treated with 50 mg/kg VE had a reduced hepatic MDA level ($p < 0.01$) in comparison to the model control mice.[27] Sizlan et al.[28] reported that oral supplementation of rats with procyanidins (consisting of catechin and epicatechin 30%, procyanidin 25%) prior to the intestinal ischemia/reperfusion (IR) injury significantly reduced the extent of lipid peroxidation in the tissue. Additionally, Roig et al.[29] showed that procyanidin (consisting of monomers 21.3%, dimers 17.4%, trimers 41.3%, and higher polymers 20%) was a powerful protective agent against H_2O_2-induced hepatocellular lipid peroxidation. In our research, PARE from sorghum bran significantly reversed the increased MDA level induced by D-galactose, suggesting that PARE was effective in scavenging the free radicals and suppressing the production of MDA.

Effect of PARE on SOD and GSH-Px Activities. The antioxidant enzymes SOD and GSH-Px represent a kind of protection against oxidative stress. These are considered as an index of antioxidant status of tissues. SOD serves as a first gatekeeper in the antioxidant defense system to scavenge superoxide anion,[30] whereas GPx is the catalyzer, which activates the reaction of lipid hydroperoxides with reduced glutathione to form glutathione disulfide.[31] As shown in Table 3, the model control mice had total SOD and GPx activities significantly ($p < 0.01$) decreased both in liver and in serum in comparison to the normal control group. The VE group had significantly ($p < 0.01$) increased total SOD and GPx activities compared to the model group. However, PARE administration at the dose of 150 mg/kg significantly ($p < 0.05$) restored (+12.80 and +6.12%, respectively) SOD activity in both liver and serum compared to the model group. Similarly, PARE had significantly increased GPx activity (+18.71 and +30.83%, respectively; $p < 0.05$) in liver and serum. However, Suwannaphet et al.[32] showed that supplementation with 0.5 and 1.0% grape seed extract (GSE, consisting of procyanidins 49.08% and monomeric flavanols 1.02%) significantly increased hepatic SOD activity (+42 and +64%, respectively), whereas it showed no significant effect on GPx activity. We inferred that the difference may result from the chemical compositions of the extracts as well as the doses used to treat the mice.

Table 4. Effect of Oral Administration PARE on Tumor Growth in C57BL/6J Mice[a]

group	treatment (mg/kg)	tumor weight (g)	inhibition (%)
control		8.6 ± 1.4	
CTX	30	1.3 ± 0.3 **	84.70
PAREL	100	7.9 ± 0.8	8.14
PAREM	200	6.5 ± 0.5 *	32.21
PAREH	400	4.1 ± 0.6 **	52.30

[a] Values are expressed as the mean ± SD for 10 mice. (*) $p < 0.05$ and (**) $p < 0.01$, compared with the control group.

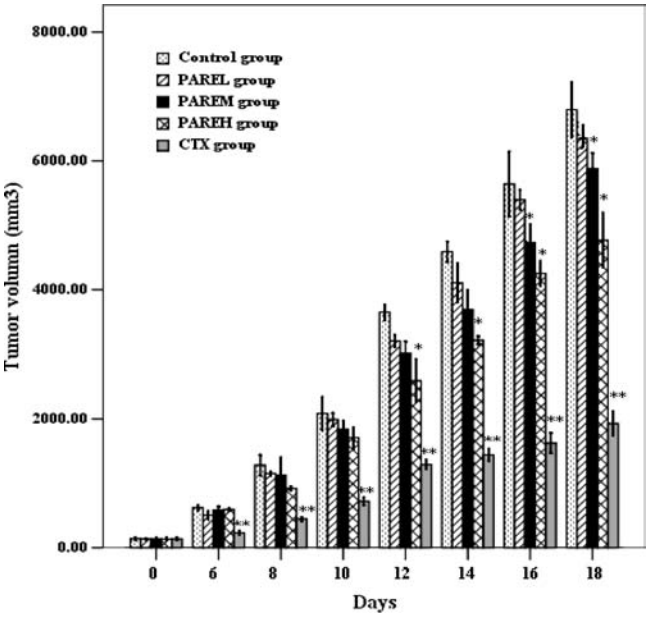

Figure 3. Effect of PARE on primary tumor growth in C57BL/6J mice. The tumor size was measured with calipers every 2 days and expressed as volume. Values are expressed as the mean ± SD. (*) $p < 0.05$ and (**) $p < 0.01$, compared to the control group.

The beneficial effect associated with supplementation of PARE at the dose of 150 mg/kg was significantly ($p < 0.05$) weak in comparison with supplementation of 50 mg/kg of VE (Table 3). We could infer that the antioxidant activity of PARE was weaker than that of VE on the same weight basis. However, PARE administration (150 mg/kg) significantly ($p < 0.05$) blocked and reversed oxidative damage in mice, suggesting that PARE possessed effective antioxidant activity and was able to prevent the D-galactose-induced oxidative stress. The antioxidant property of PARE might contribute to its further exploration as an antioxidant functional food in the prevention of aging-related diseases.

Effects of PARE on Tumor Growth and Metastasis Formation in C57BL/6J Mice. To determine the effects of PARE on tumor growth and metastasis formation, the standard reference drug (CTX) and three doses of PARE were administered, respectively. Compared to the control group, PARE-treated groups at doses of 100, 200, and 400 mg/kg BW, respectively, showed inhibition of tumor growth throughout the study (Table 4; Figure 3). There was significant ($p < 0.05$) inhibition on tumor volume in the PAREM group (200 mg/kg) and PAREH group (400 mg/kg) as compared to the control group,

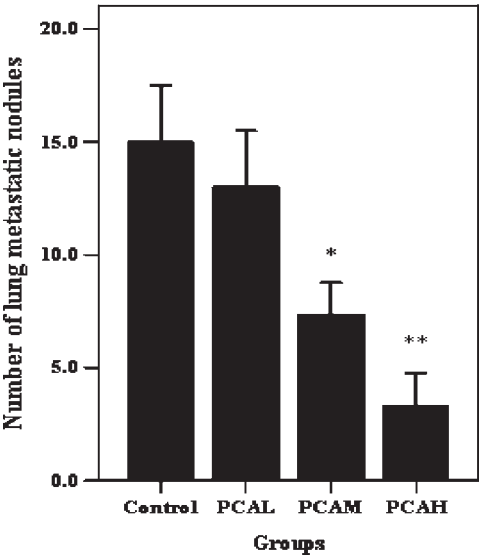

Figure 4. Effect of PARE on Lewis lung cancer metastasis in C57BL/6J mice. Values are expressed as the mean ± SD, $n = 10$ mice. (*) $p < 0.05$ and (**) $p < 0.01$, compared with the control group.

although significant ($p < 0.01$) inhibition was found in CTX group. Similarly, compared to the control group, administration of PARE at doses of 100, 200, and 400 mg/kg resulted in inhibition of tumor weight coupled with inhibition rates of 8.14%, 32.21% ($p < 0.05$), and 52.30% ($p < 0.01$), respectively. The results were in accordance with the study of Mantena et al., who demonstrated that administration of 0.2 and 0.5% grape seed procyaindins (consisting of dimers 6.6%, trimers 5.0%, tetramers 2.9%, and other oligomers 74.8%), in high-risk groups, resulted in 30 and 52% inhibition in tumor volume, respectively. Similarly, 21 and 49% reductions were also found in tumor weight compared to mice not fed grape seed procyanidins at the termination of the experiment.[33]

For all of the groups, metastases on the lung surfaces were calculated, and the results are shown in Figure 4. Significant inhibition of metastasis formation was found in the PAREM group ($p < 0.05$) and the PAREH group ($p < 0.01$) compared to the control group. However, no metastasis was found in the CTX group. Our results suggested that PARE could effectively suppress tumor growth and metastasis formation in C57BL/6J mice bearing Lewis lung cancer. Previous study showed that *Pinus koraiensis* extract (procyanidins 21.04%) had a dose-dependent antitumor activity on mice bearing U14 cervical cancer and indicated that procyanidins possessed a direct effect of killing and wounding tumor cells.[34] Procyanidins may mediate apoptosis in cells via the mitochondrial disruption pathway and activation of caspase 3. It was known that apoptosis plays a crucial role in eliminating the mutated neoplastic and hyperproliferating cells from the system and, therefore, may be considered as a protective mechanism against cancer progression.[33]

Effect of PARE on VEGF Production. To assess the effect of PARE administration on VEGF expression levels in vivo, embedded tumor sections were analyzed by immunohistochemical staining for VEGF using specific antibodies. Representative images of tumor sections analyzed for VEGF are shown in Figure 5, panels A, B, C, D, and E, respectively. Microscopic examination of VEGF-stained tumor sections showed decreases in the intensity of VEGF-positive cytoplasmic staining in PARE-fed

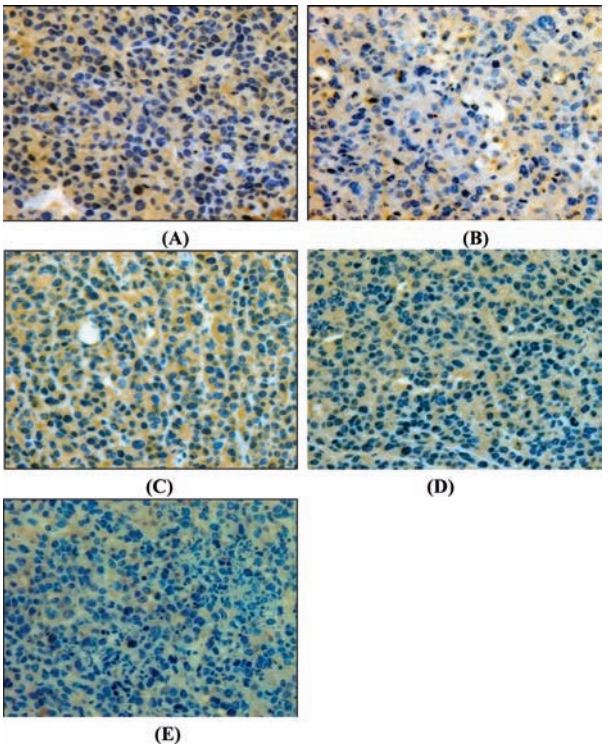

Figure 5. Effect of PARE on VEGF production in C57BL/6J mice: (A) control group; (B) CTX group; (C) PARE low-dose group; (D) PARE middle-dose group; (E) PARE high-dose group (HE ×200).

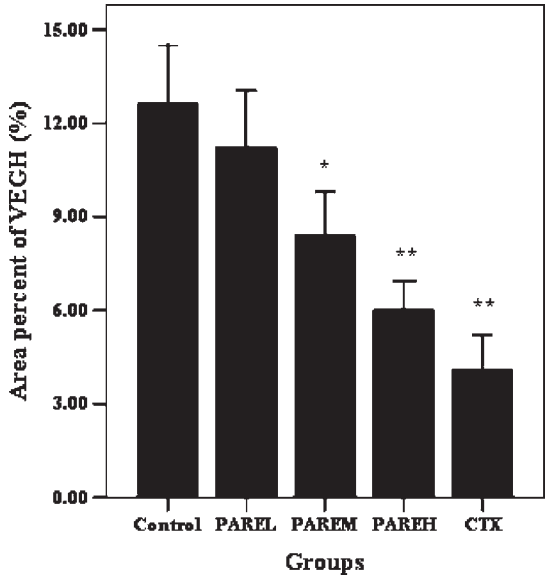

Figure 6. Area percent of VEGF in mice with different treatments. Data are expressed as the mean ± SD. (*) $p < 0.05$ and (**) $p < 0.01$, compared with the control group.

groups compared to the control group, and the results are summarized in Figure 6. Compared to the control group, VEGF production was significantly decreased in PAREM (−33.3%, $p < 0.05$) and PAREH (−52.4%, $p < 0.01$) groups; however, no significant difference was found in the PAREL group. Whereas Lu et al. reported that grape seed extract (50 mg/kg), constituting of at least 85% procyanidins, could significantly reduce VEGF production (−72%) in U251 cells when compared to the control cells.[35]

Angiogenesis, the formation of new blood vessels, plays a critical role in tumor progression.[36,37] There are multiple factors involved in tumor angiogenesis. However, VEGF is one of the most critical and specific angiogenesis factors.[38] Here, we show that PARE administration can decrease VEGF production in mice. Procyanidins are widely consumed as a dietary supplement and possesse anticancer activity against various cancers.[33,34] Previous study showed that oligomeric procyanidins (predominately trimers and tetramers) derived from cinnamon extract could inhibit angiogenesis by blockingVEGFR2 kinase and downstream signal.[39] The inhibitory effect of procyanidins on VEGF production may contribute to the inhibitory transcriptional activation of the VEGF gene through reducing protein but not mRNA expression of HIF-1α.[35] Procyanidins from various sources possessed inhibitory activity of VEGF, and by far it is the most potent effect on endothelial function compared to other phenolic compounds.[40] In the present study, PARE-induced decrease in VEGF production might play an important role in the inhibition of tumor angiogenesis, growth, and metastasis. However, further study is needed to determine the precise molecular mechanism of anticancer activity of PARE from sorghum bran.

In summary, the present study shows that procyanidin-rich extract (PARE) from sorghum bran significantly ($p < 0.05$) repairs the oxidative damage in mice by increasing the activities of SOD and GSH-Px in both liver and serum. Moreover, PARE effectively inhibits tumor growth and metastasis formation by suppression of VEGF production in vivo. Our results suggest that PARE, as a natural effective antitumor agent, has the potential to be employed as a routine diet-based strategy for cancer prevention or treatment.

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Author Contributions

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ABBREVIATIONS USED

PARE, procyanidin-rich extract; FLD, fluorescence detectors; DP, degree of polymerization; MDA, malondialdehyde; GSH-Px, glutathione peroxidase; SOD, superoxide dismutase; VEGF, vascular endothelial growth factor; VE, vitamin E; CTX, cyclophosphamide; ROS, reactive oxygen species; PAREL, PARE

low-dose group; PAREM, PARE middle-dose group; PAREH, PARE high-dose group; PBS, phosphate-buffered saline.

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