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Chinese Yam (*Dioscorea alata* cv. Tainung No. 2) Feeding Exhibited Antioxidative Effects in Hyperhomocysteinemia Rats

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Antioxidative effects of *Dioscorea alata* (*D. alata*) were investigated in hyperhomocysteinemia (HHcy) induced by methionine (Met) oral feeding (1 (g/kg of BW)/day). HHcy rats were fed a standard laboratory chow supplemented without or with freeze-dried *D. alata* powder at 1, 2.5, and 5 (g/kg of BW)/day, assigned as Met, Met + D1, Met + D2, and Met + D3 groups, respectively. Twelve weeks after *D. alata* feeding, plasma homocysteine levels (16.3−24.2 μM) were significantly decreased compared to that of the Met group (34.1 ± 9.9 μM) ($p < 0.01$), and similar to the basal level (15.0 ± 1.9 μM). Thrombin-induced platelet aggregation (PA) of the Met + D2 and Met + D3 groups was significantly lower than that of the Met group. Plasma malondialdehyde levels, an indicator of lipid peroxidation, and hepatic reactive oxygen species, an indicator of oxidative stress, of HHcy with *D. alata* feeding were significantly lower than that without *D. alata* feeding. The hepatic catalase in the Met + D2 and Met + D3 groups was significantly elevated compared to that in the Met group. *D. alata* feeding did not significantly change hepatic superoxide dismutase, glutathione peroxidase, and glutathione reductase, which were adaptively enhanced by Met feeding. The decreased glutathione/glutathione disulfide ratio in the Met group was increased after *D. alata* feeding. These results indicated that HHcy induced by Met could be reversed by *D. alata* feeding. *D. alata* feeding exhibited its antioxidative effects in HHcy including alleviating PA, lipid peroxidation, and oxidative stress, but did not induce activity of antioxidant enzymes which had already adaptively increased by HHcy.

KEYWORDS: ***Dioscorea alata*; yam; oxidative stress; homocysteine; hyperhomocysteinemia; antioxidative activity; reactive oxygen species; superoxide dismutase; catalase; glutathione peroxidase; glutathione reductase**

INTRODUCION

Different yam species of *Dioscorea* have been widely used for enhancement of health in Eastern countries and listed as a superior medical herb substance and functional food for traditional treatment of several illnesses in China (*1*). However, its scientific basis as a functional food is not understood. Recently, the concept of functional food raises the need for scientific evaluation of the benefit or risk of these traditional herb plants (*2*, *3*). *Dioscorea alata* cv. Tainung No. 2 was released by the Taiwan Agricultural Research Institute (TARI) for commercial production in 1996. It is one of the most popular and widely cultivated yams in Taiwan due to its superior characteristics such as high nutrient value, resistance against anthracnose, high and stable yield potential, and wide adaptability (*4*). Despite an increasing interest in this yam, published data on the antioxidative effect of the locally grown yam are scanty.

Oxidative stress, including high concentrations of free radicals and reactive oxygen species (ROS), is able to start adverse chain reactions such as lipid peroxidation and damage to cellular lipids, proteins, carbohydrate, and DNA. Thus, it has been implicated as a contributing factor to aging and progression of chronic diseases such as cancer and cardiovascular disease (CVD) (*5*−*8*).

Elevated plasma homocysteine (Hcy) is considered to be a risk factor for CVD (*9*, *10*), although its mechanism of action is not completely clear. The thiol (−SH) group of Hcy is readily oxidized, and during oxidation, superoxide anion radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^-) are generated. These oxygen-derived molecules are believed to account for Hcy-induced oxidative stress (*11*). $O_2^{\bullet -}$ and OH^- generated during oxidation of Hcy initiate lipid peroxidation. Hyperhomocysteinemia (HHcy) due to oral methionine (Met) loading induced an increase in plasma oxidation markers and a decrease in antioxidant capacity in human (*12*). Excessive free radical and ROS production leads to oxidative capacity in human (*12*). Excessive free radical and ROS production leads to oxidative stress that can cause cell damage (*13*). Therefore, prevention of oxidative stress caused by HHcy can play a

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significant role in good health and prevention of disease due to the high protein (Met) intake.

The antioxidative activity of *Dioscorea* is still unknown, although it has been reported in some species. A crude enzyme from *Dioscorea esculenta* revealed polyphenol oxidase (PPO) and peroxidase (PO) activities (*14*). PPO and PO activities were also found in some *Dioscorea* genotypes (*15*). Dioscorin, the storage protein of yam (*Dioscorea batatas*) tuber, had scavenging activities against both 1,1-dipheny1-2-picrythydrazyl 1 (DPPH) and hydroxyl radical (*16*). Mucilage of yam (*D. batatas*) exhibited antiradical (DPPH and hydroxyl radical) and antioxidant activities in vitro (*17*). Yam (*D. alata*) mucopolysaccharide significantly increased the myeloperoxidase and enhanced oxidative response (H_2O_2, $O_2^{\bullet -}$, NO) and cytokine production (tumor necrosis factor) in murine peritoneal macrophage (*18*). After young mice were fed a 20% water crude extract of *Dioscorea rhizoma* for 6 weeks, plasma glutathione peroxidase (GSHPx) increased, and lipid peroxide (LPO) decreased (*19*). Three weeks of supplementation with commercial *Dioscorea* capsules resulted in significant antioxidant activity to modify serum lipid levels, including lowered triglycerides, LDL cholesterol, and lipid peroxidation in the elderly (*20*). However, the antioxidant effect of *D. alata* on the HHcy induced by Met has not yet been reported.

In the present study performed with rats, we have set up a dietary-induced HHcy with a Met oral feeding (1 (g/kg of BW)/day). Then a freeze-dried powder of *D. alata* was given to HHcy rats to determine if a hypo-Hcy effect, less oxidative stress, and lipid peroxidation occurred.

MATERIALS AND METHODS

Animals and Treatment. Male Spraque−Dawley rats, weighing 150−200 g, 6 weeks old, were purchased from the Animal Center (Medical College, National Cheng Kung University, Tainan, Taiwan) and housed individually in a well-ventilated room maintained at 22 °C, on a 12 h light−dark cycle. They were pair-fed weekly with 100 g of food per rat and were provided free access to water. HHcy was induced by Met oral feeding (1 (g/kg of BW)/day) for 10 weeks. HHcy in the rats was confirmed by measurement of the plasma Hcy level and randomly divided into five groups: the Met feeding only (Met), Met with 200 (mg of vitamin C/kg of BW)/day (Met + vitC), and Met with freeze-dried *D. alata* powder at 1, 2.5, and 5 (g/kg of BW)/day (Met + D1, Met + D2, and Met + D3, respectively). *D. alata* powder was made from fresh yam (*D. alata* cv. Tainung No. 2) which was provided by TARI (Council of Agriculture, Executive Yuan, Taichung, Taiwan) and freeze-dried prior to being ground into a powder. Analyses were performed after 12 weeks of *D. alata* feeding.

Homocysteine Measurements. Plasma levels of Hcy were assayed on the basis of HPLC of the fluorescent 7-benzo-2-oxa-1,3-diazole-4-sulfonic acid (SBD-F) derivative (*21*). The derivatization of plasma Hcy was performed with the Ubbink method (*22*). In summary, the plasma samples (200 μL) were supplemented with an internal recovery standard, *N*-acetylcysteine (NAC) (1.1 mM, 20 μL), treated with tri-*n*-butylphosphine (TBP) solution (100 g/L in dimethylformamide, 20 μL), and incubated at 4 °C for 60 min before precipitation with 10% TCA. A 100 μL sample of supernatant was obtained by centrifugation, incubated with borate/EDTA buffer, NaOH solution, and SBD-F solution for 60 min, 60 °C, and then cooled to room temperature. All derivatized samples were filtered (0.22 μm) and injected (20 μL) onto a reversed-phase HPLC column (Merck LiChrospher RP-18e 250-4mm, 5 μm). With a Hitachi L-7100 pump, isocratic elution occurred at 1.2 mL/min with potassium phosphate (K-P) buffer (0.1 mol/L) containing 35 mM CH_3CN, pH 3.5. Fluorescence detector (F-1050, Hitachi, Tokyo, Japan) excitation and emission wavelengths were 385 and 515 nm. Data were analyzed with a SISC-LAB data processor. Recovery of Hcy was 91.56% ± 1.45%. Within- and between-assay reproducibilities were 6.36% and 8.29%, respectively.

Platelet Aggregation Analysis. The blood was anticoagulated with a 3.8% sodium citrate solution (9:1 v/v) and centrifuged at 240*g* for 10 min, and the supernatant was aspirated to procure platelet-poor plasma (PPP). The concentration of platelets in PRP was diluted to $(2-3) \times 10^6$ platelets/mL with PPP for platelet aggregation (PA). Cuvettes containing 450 μL of PRP were placed in a turbidimetric aggregometer (Helena platelet aggregation chromogenic kinetic system-4, Beaumont, TX) under stirring at 37 °C. Aggregation was initiated by the addition of thrombin (3.2 U/mL). The extent of aggregation was expressed as a percentage of the maximum change in optical density represented by autologous PPP.

Thiobarbituric Acid Reactive Substance Measurements. Plasma lipid peroxidation was measured as thiobarbituric acid reactive substances (TBARS) expressed in malondialdehyde (MDA) equivalents (*23*). In summary, 100 μL of the plasma samples was treated with 0.2 mL of 8.1% sodium sodecyl sulfate (SDS), 1.5 mL of 20% acetic acid solution, and 1.5 mL of an 0.8% aqueous solution of thiobarbituric acid (TBA). The mixture was increased to 4.0 mL using distilled water, and then heated at 95 °C for 60 min. After the mixture was cooled to room temperature, an equal volume of 1-butanal was added, and the mixture was shaken vigorously. After centrifugation at 1500*g* for 10 min, the fluorescence intensity of the organic layer (upper layer) was measured at excitation and emission wavelengths of 515 and 553 nm, respectively.

Reactive Oxygen Species Measurements. ROS generation was monitored by a chemiluminescent technique modified from Barroso et al. (*24*) using luminal (5-amino-2,3-dihydro-1,4-phalazinedione) as the probe. In summary, 1 mg of liver was homogenized in 0.3 mL of 50 mM sodium phosphate (Na-P) buffer and exposed to 50 μL of a 10 μM luminal solution in DMSO. Chemiluminecsence was monitored as counts integrated over a 1 s period in a Lumat LB9507 luminometer (Lumat LB9507, EG&G, Berthold, Germany). Results were expressed as the difference of counts between post-luminol and pre-luminol (basal) conditions.

Vitamin C Measurements. Plasma levels of vitamin C were assayed on the basis of HPLC modified from Rose et al. (*25*). The blood was anticoagulated with a 3.8% sodium citrate solution (9:1 v/v), and centrifuged at 2400*g* for 10 min. The plasma samples (250 μL) were added to an equal volume of 10% metaphosphoric acid. This mixture was vortexed briefly and centrifuged for 10 min at 1000*g* at 4 °C. The supernatant was passed through a Millipore filter (0.22 μm) and injected (20 μL) onto a reversed-phase HPLC column (Hypersil HS C18, 250 × 4.6 mm, 5 μm, Thermo Quest, England). With a Jasco PU-1580 pump, isocratic elution occurred at 0.7 mL/min with 1-pentanesulfuric acid sodium salt (PICB, 0.871 g/L), at pH 3.1. The UV detector (UV-1575, Jasco, Tokyo, Japan) wavelength was 254 nm. Data were analyzed with a Chrom Manager (Analab Corp., ROC) data processor. Recovery of vitamin C was 94.72% ± 3.41%. Within- and between-assay reproducibilities were 2.15% and 4.41%, respectively.

Superoxide Dismutase Measurements. Superoxide dismutase (SOD) was assayed by a modification of the method of Marklund and Marklund (*26*). One unit of SOD activity was defined as the amount of the enzyme inhibiting the autoxidation by 50%. In summary, 0.3 g of liver and Na-P buffer (50 mM, 0.5 mL) were homogenized. The homogenized liver (200 μL) was treated with 280 μL of 50 mM Tris−HCl buffer (pH 8.2) and 480 μL of 2% Triton X-100. The mixture was centrifuged for 5 min at 3000*g* at 4 °C. The supernatants were diluted with 50 mM Na-P buffer. The final assay mixture contained 10 μL of the diluted tissue supernatant, 3 mL of Tris−HCl buffer, and 6.1 μL of the pyrogallol (50 mM in 10 mM HCl). The solution was assayed immediately to monitor the change of absorbance measured within 1 min at 325 nm using a spectrophotometer (DU 530, Beckmen, Fullerton, CA).

Catalase Measurement. Using the method of Aebi (*27*), the oxidation of H_2O_2, which acts as a substrate, was catalyzed by catalase to produce H_2O and O_2. In the presence of catalase, the absorbance of H_2O_2 was gradually decreased ($\epsilon = 240 = 40$ cm^2/μmol). The catalase activity was determined by the change of absorbance within a unit of time. In summary, 0.5 mL of 50 mM Na-P buffer (pH 7.4) was added to each 0.3 g liver sample for homogenizing. A 200 μL sample of the homogenate was added to 280 μL of 50 mM K-P buffer (pH 7.0) and

480 μL of Triton X-100 (2%). These were then centrifuged at 3000*g* for 5 min, 4 °C. The supernatants were diluted with 50 mM of K-P buffer. Tissue supernatant diluent (2 mL) was added to 1 mL of H_2O_2 (30 mM), they were mixed well, and the mixture was assayed immediately to monitor the change of absorbance within 1 min at 240 nm using a spectrophotometer (DU 530, Beckmen).

Glutathione Peroxidase and Reductase Measurement. Glutathione peroxidase (GSHPx) and reductase (GSSGR) were assayed by a modification of the methods of Paglia and Valentine (*28*) and Bellomo et al. (*29*), respectively, in which GSHPx catalyzes the oxidation using cumene hydroperoxide as the substrate. Oxidized glutathione is then used as a substrate for GSSGR, with the subsequent oxidation of added NADPH monitored as a decrease in absorbance at 340 nm. The liver preparation was as described above for catalase measurement. The tissue supernatants were diluted with 0.25 M K-P buffer. For GSHPx assay, 100 μL of the tissue supernatant diluent was added to 200 μL of GSSGR (5 units/mL), 50 μL of glutathione (40 mM), and 620 μL of K-P buffer (0.25 M). To this mixture were added 10 μL of 20 mM NADPH (in 1% Na_2CO_3) and 20 μL of 15 mM cumene hydroperoxide. For GSSGR assay, 150 μL of the tissue supernatant diluent was added to 300 μL of 3.7 mM GSSG and 50 μL of 20 mM NADPH (in 1% NA_2CO_3). The solution was assayed immediately to monitor the change of absorbance within 1 min at 340 nm using a spectrophotometer (DU 530, Beckmen).

Protein Assay. Protein was measured using the BCA protein assay kit (Pierce, Rockford, IL). All enzyme activities were expressed as units per milligram of protein.

Glutathione and Glutathione Disulfide Measurements. Glutathione (GSH) and glutathione disulfide (GSSG) were assayed by a modification of the method of Hissin and Hilf (*30*). In summary, 250 mg of homogenized liver sample in 4.75 mL of 50 mM phosphate buffer was centrifuged for 30 min at 100 000*g* at 4 °C. To 0.5 mL of the supernatant, 4.5 mL of the 50 mM Na-P buffer (pH 8.0) was added for GSH assay. Another 0.5 mL of the supernatant was incubated at room temperature with 200 μL of 0.04 M *N*-ethylmaleimide (NEM) for 30 min. To this mixture was added 4.3 mL of 0.1 N NaOH for GSSH assay. Each final assay mixture (2.0 mL) contained 100 μL of the diluted tissue supernatant, 1.8 mL of phosphate buffer, and 100 μL of *o*-phthalaldehyde (OPT) containing 100 μg of OPT. After thorough mixing and incubation at room temperature for 15 min, the fluorescence intensity of the solution was measured at an excitation wavelength of 350 nm and an emission wavelength of 420 nm with a fluorescence spectrophotometer (F-4500, Hitachi, Tokyo, Japan).

Statistical Analysis. Unless stated otherwise, data were presented as mean ± SD and analyzed by SAS within the groups. Data from the different groups were compared by the one-way ANOVA method followed by Duncan's test (when $p < 0.05$).

RESULTS AND DISCUSSION

Plasma Hcy levels were significantly increased 3-fold from 6.7 ± 1.0 (basal) to 21.7 ± 4.5 μM after 10 weeks of Met feeding, indicating that a model of dietary HHcy could be induced by oral Met feeding in rats. Twelve weeks after *D. alata* feeding, plasma Hcy levels of Met + D1, Met + D2, and Met + D3 were significantly lower than that of the Met group and similar to those of the Met + Vit C and control groups (**Figure 1**). This result demonstrated that *D. alata* feeding regulated the levels of Hcy in HHcy rats down to that in the control. The efficiency of *D. alata* feeding in the regulation of Hcy level was similar to that of vitamin C feeding in an amount of 200 (mg/kg of BW)/day, which was used as a positive control to test the efficiency of the antioxidative effect of *D. alata* in this study since vitamin C has been reported to be a potent antioxidant scavenger of ROS (*31*). Plasma levels of vitamin C were found to be negatively correlated with both Hcy and lipid peroxide (*32*). In addition, oral administration of antioxidant vitamin C (2 g) was found to prevent endothelial dysfunction of Met-induced HHcy (*33*). Pretreatment with vitamin C (1 g/day, for 1 week) could prevent the impairment of vascular endothelial function that is associated with an elevation in Hcy concentration induced by oral Met (100 mg/kg) feeding, indicating that the adverse effects of Hcy are mediated through an oxidative stress mechanism (*34*). However, the antioxidant substance in *D. alata* needs to be further determined since the plasma vitamin C level was not significantly elevated after 12 weeks of *D. alata* feeding in this study. Thus, the mechanism of *D. alata* in reducing the plasma level of Hcy in this study may be attributed to its other antioxidant activity found in many different *Dioscorea* species, such as PPO, PO, dioscorin, mucilage, and mucopolysaccharide (*14*−*20*).

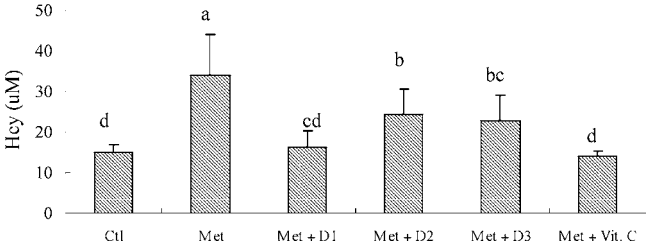

Figure 1. Plasma homocysteine levels of hyperhomocysteinemia rats induced by Met feeding and fed with *D. alata* at 1.0 (D1), 2.5 (D2), and 5 (D3) (g/kg of BW)/day for 12 weeks. Means ($n = 6$) without a common superscript letter are significantly different from each other ($p < 0.01$).

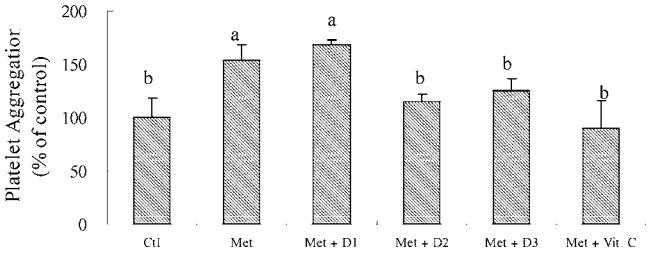

Figure 2. Thrombin-induced platelet aggregation of hyperhomocystemia rats induced by Met feeding and fed with *D. alata* at 1.0 (D1), 2.5 (D2), and 5 (D3) (g/kg of BW)/day for 12 weeks. Means ($n = 6$) without a common superscript letter are significantly different from each other ($p < 0.01$).

Thrombin-induced PA of Met + D2 and Met + D3 was significantly lower than that of the Met group (**Figure 2**). No significant differences were found between the PA of *D. alata* feeding (Met + D2 and Met + D3 groups) and that of both Met + Vit C and the control groups. The potential for Hcy-induced platelet hyperactivity (*35*, *36*) was more strengthened by a dose−response study of Met load, indicating that a higher plasma Hcy concentration is linked to enhance thrombin- and ADP-evoked PA (*37*).

Plasma levels of MDA, an indicator of lipid peroxidation, in Met + D1, Met + D2, and Met + D3 were significantly lower than that in the Met group and similar to that in both Met + Vit C and control groups (**Figure 3**). Elevated plasma Hcy levels in conjunction with increased plasma lipid peroxidation after oral Met feeding in our study agree with the results of other studies indicating a significant and strong positive correlation between Hcy and lipid peroxidation (*12*, *32*, *38*). However, the relationship between Hcy and MDA, which is widely used as an index of oxidative damage (*39*, *40*), is still controversial. Some authors have reported no change in the Hcy level and the reaction with thiobarbituric acid in healthy volunteers after the first administration of Met or after 1 month of treatment (250 mg four times daily) (*41*). No correlation was also found between Hcy and MDA concentrations, although a moderate increase of Hcy is associated with CVD and lipid peroxidation

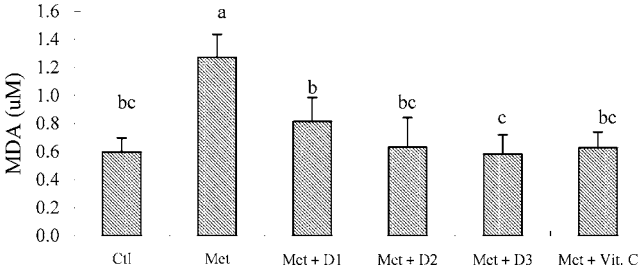

Figure 3. Plasma MDA levels of hyperhomocysteinemia rats induced by Met feeding and fed with *D. alata* at 1.0 (D1), 2.5 (D2), and 5 (D3) (g/kg of BW)/day for 12 weeks. Means ($n = 6$) without a common superscript letter are significantly different from each other ($p < 0.01$).

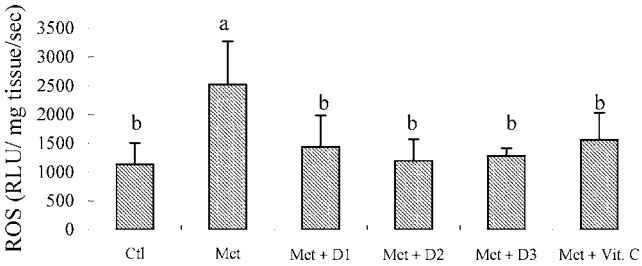

Figure 4. Hepatic ROS levels of hyperhomocysteinemia rats induced by Met feeding and fed with *D. alata* at 1.0 (D1), 2.5 (D2), and 5 (D3) (g/kg of BW)/day for 12 weeks. Means ($n = 6$) without a common superscript letter are significantly different from each other ($p < 0.01$).

is shown by the increased plasma free and total MDA concentrations in CVD (*42*). It appears that the correlation between Hcy and MAD occurs only at high Hcy concentrations (54.3 μM) after Met loading (*12*). Nevertheless, feeding *D. alata* at 1.0, 2.5, and 5 (g/kg of BW)/day for 12 weeks in the present study significantly decreased the plasma levels of MDA to that of both the control and Met + Vit C groups, indicating the antioxidative effect of *D. alata.*

Hepatic ROS, an indicator of oxidative stress, of Met + D1, Met + D2, and Met + D3 were significantly lower than that of the Met group (**Figure 4**). No significant differences were found between the hepatic ROS of *D. alata* feeding (Met + D1, Met + D2, and Met + D3 groups) and both control and Met + Vit C groups. The hepatic ROS level increase in Met feeding rats with high levels of Hcy in our study confirmed another study in which ROS release was increased in rabbit aortic endothelial cells incubated with Hcy (*43*). *D. alata* feeding (Met + D) significantly decreased the hepatic ROS levels, indicating that Met-induced HHcy responsible for the production of ROS was reversed by *D. alata* feeding in this study. It has been suggested that HHcy may promote the production of hydroxyl radicals, known lipid peroxidation initiators, through Hcy autoxidation and thiolactone formation (*44*, *45*).

Hepatic antioxidant enzyme activities, including SOD, GSHPx, and GSSGR of Met, Met + *D. alata*, and Met + Vit C feeding, were not significantly different from one another, but significantly higher than that of the control, which was not fed with Met. Studies demonstrated that there must be a compensatory mechanism to increase the level of antioxidant enzyme activities in response to oxidative stress such as high concentrations of glucose (*46*) and hyperoxia (*47*). The information regarding the activities of antioxidant enzymes in response to HHcy was not clear. Elevated plasma Hcy leading to an adaptive increase in the activities of antioxidant enzymes (erythrocyte SOD and GSHPx and plasma GSHPx) in circulation was found in patients with inherited defects of Hcy metabolism (*48*). No increase in erythrocyte catalase (*47*, *48*) or GSHPx (*48*) activity was found in HHcy patients. Rabbits fed a Met-rich diet for 9 months elicited an increase in erythrocyte SOD activity but a concomitant fall in erythrocyte catalase and GSHPx activity (*49*). The decreased activity of GSHPx was found in endothelial (*50*) and smooth muscle (*51*) cell culture in a dose-dependent manner with increasing Hcy levels, although an increase in SOD activity was found in smooth muscle cell with increasing Hcy levels (*52*). The increased activities of hepatic SOD, GSHPx, and GSSGR in Met feeding in the present study indicated an adaptive increase in the activities of antioxidant enzymes in liver, which is one of the very active sites for Hcy metabolism, representing protective antioxidant response to HHcy-induced oxidative stress. Therefore, in Met-induced HHcy, autoxidation of Hcy, with concomitant generation of ROS, may also induce the up regulation of antioxidant enzymes. Thus, *D. alata* feeding did not further enhance the activities of hepatic SOD, GSHPx, and GSSGR in this study.

Hepatic catalase activity was not significantly elevated in the Met group, indicating that Met-induced HHcy did not induce catalase activity. Hepatic catalase activities of Met + D2 and Met + D3 were significantly elevated compared to that of the Met group, which was not fed with *D. alata.* Catalase activity was not induced by Met feeding in the present study, suggesting that Met-induced HHcy may increase $O_2^{\bullet -}$ primarily. The enhanced catalase activity in the middle (D2) and high (D3) doses of *D. alata* feeding indicate a protective effect of *D. alata* in the case of no adaptive increase in activity of catalase in HHcy.

After 12 weeks of *D. alata* feeding, the hepatic GSH/GSSG ratio was significantly decreased in Met (2.5 ± 1.1) compared to that in the control (6.1 ± 3.3) groups. HHcy rats fed with *D. alata* (Met + D2 and Met + D3) had significantly increased GSH/GSSG ratios (6.8 ± 0.8 and 4.1 ± 1.4, respectively) compared to that of the Met group. A decreased GSH/GSSG ratio, an indicator of antioxidative defense (*52*), found in the Met feeding was increased after *D. alata* feeding in the present study, indicating that *D. alata* feeding favors the antioxidative state via the redox regulation in HHcy. Additionally, the increased GSH/GSSG ratio in the *D. alata* feeding may contribute to the deceased plasma Hcy (*53*) in HHcy induced by the Met feeding.

ABBREVIATIONS USED

CAT, catalase; D, *Dioscorea alata*; GSH, glutathione; GSHPx, glutathione peroxidase; GSSG, glutathione disulfide; GSSGR, glutathione reductase; Hcy, homocysteine; HHcy, hyperhomocysteinemia; MDA, malondialdehyde; Met, methionine; PA, platelet aggregation; ROS, reactive oxygen species; SOD, superoxide dismutase; Vit C, vitamin C.

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