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Research Article

Potential Effects of Antioxidant and Serum Cholesterol-Lowering Effects of *Gynura bicolor* Water Extracts in Syrian Hamster

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Gynura bicolor (Roxb. and Willd.) DC (*G. bicolor*) is a dietary vegetable in the Far East. The aims of the present study were to investigate the antioxidant effects of the *G. bicolor* water extract (GBWE) and its ability to regulate the blood lipid and lipoprotein profiles. In this study, the pigment composition and antioxidant ability of the GBWE were analyzed. Syrian hamsters were fed a high-fat diet (HFD) and the GBWE for 12 weeks, and the blood lipid levels, lipoprotein profiles, and cholesterol metabolism-related enzyme levels were then examined. The results showed that the GBWE exhibited excellent 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity and ferrous-ion-chelating ability. The hepatic glutathione levels in the hamsters were increased after the administration of low (0.4 g/kg BW, GBWE-L) or high (0.8 g/kg BW, GBWE-H) levels of the GBWE. The GBWE-H-treated hamsters exhibited significantly decreased serum levels of total cholesterols (TC) and low-density lipoprotein-cholesterol (LDL-C) and significantly increased levels of lectin-cholesterol acetyltransferase (LCAT). These results showed that GBWE-H can reduce the total cholesterol and LDL-C levels in HFD-fed hamsters, and this reduction might be involved in the regulation of LCAT expression.

1. Introduction

Gynura bicolor (Roxb. and Willd.) DC (*G. bicolor*) is a vegetable and traditional herb in Taiwan and other eastern Asian countries. Both sides of the leaves of *G. bicolor* are dark green and purple, which gives this plant a unique outward appearance. Lu et al. [1] demonstrated that flavonoids might be the major constituents of *G. bicolor* that give the leaves their unique colouring and might underlie the beneficial physiological effects of this herb. Previous studies have shown that *G. bicolor* exhibits neuronal protective [2], blood glucose-lowering [3], liver-protective [4], antioxidant [5], and anticolorectal cancer properties [6], and our previous studies revealed that *G. bicolor* can exert anti-inflammatory effects [7] and promote iron bioavailability [8]. However, to the best of our knowledge, the effects of *G. bicolor* on the blood lipid levels and lipoprotein profiles have not been reported.

Cardiovascular disease is one of the most common causes of death worldwide [9]. Disorders in the blood lipid levels and lipoprotein profiles are the primary causes of cardiovascular diseases [10]. Elevated levels of free radicals and lipid peroxides cause oxidative stress and induce degenerative diseases, such as cardiovascular disease [11]. In a randomized controlled trial, Xie et al. [12] showed that dietary polyphenols reduce the levels of biomarkers associated with an increased risk of cardiovascular disease, including total triglycerides (TG), total cholesterol (TC), and low-density lipoprotein-cholesterol (LDL-C) levels. Phytochemicals, such as flavonoids, phenolic compounds, and anthocyanidin, which are present in *G. bicolor*, have various beneficial properties, including antioxidant effects. Our previous study showed that *G. bicolor* contains important plant pigments and active molecules, such as chlorophyll, gallic acid, and β-carotene [8]. However, the in vitro and in vivo effects of *G. bicolor*

on the blood lipid levels and lipoprotein profiles have not been determined.

The aim of the present study was to investigate the antioxidant and blood lipid-regulatory abilities of *G. bicolor* in vitro and in vivo. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical- and superoxide radical-scavenging abilities, the reducing power, and the ferric iron-chelating capacity were considered indicators of the antioxidant ability of *G. bicolor* in vitro. Hamsters were fed a high-fat diet (HFD) and administered *G. bicolor* for 12 weeks, and the hepatic glutathione (GSH) levels, thiobarbituric acid reactive substance (TBARS) levels, serum TC and TG levels, lipoprotein blood lipid profiles, and expression levels of hepatic 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMG-CoA reductase), acyl-coenzyme A:cholesterol acyltransferase (ACAT), and lecithin-cholesterol acyltransferase (LCAT) were then assessed. The results obtained in the present study highlight the potentially beneficial antioxidant effects of *G. bicolor* on the blood lipid and lipoprotein levels and cholesterol-related lipoprotein metabolism.

2. Materials and Methods

2.1. Preparation of the G. bicolor Water Extract (GBWE). *G. bicolor* was purchased from The Agricultural Production and Marketing Group of Yuanshan Village. The same plant was identified by Dr. Yen Hsueh Tseng, and a plant is being grown at the Department of Forestry, National Chung Hsieh University (NCHU). A voucher specimen (TCF13549) has been deposited at NCHU. The leaves of *G. bicolor* were removed, cleaned, and blended in cold water (4°C; w/w, 1/1). The homogenates were extracted as the mixture was stirred for 1 h on a stir plate, and the extracts were centrifuged at 250 × g and 4°C for 10 min. The supernatant was filtered and concentrated with a rotary vacuum dryer (55 C), and the concentrated product was dried in a freeze dryer at −43°C. The percentage yield of the GBWE was 4.2% (w/w).

2.2. Analysis of Chemical Components of the Major Plant Pigments from the GBWE. Plants contain four major pigments, flavonoids, carotenoids, chlorophyll, and betalains [13, 14], and among these plant pigments, we selected one or more components from each class pigment, including chlorophyll, gallic acid, and β-carotene, for the analysis in this study [8]. Furthermore, some flavonoids, including anthocyanidin, myricetin, morin, quercetin, and rutin, were analyzed in the GBWE. Specifically, the anthocyanidin, myricetin, and morin contents were analyzed using the method described by Novak et al. [15] with some modifications to the sample preparation and quantification protocols. The quercetin content was evaluated as described by Wang and Morris [16], and the rutin content was determined using the method described by Krizman et al. [17] with some modifications.

The levels of anthocyanidin, myricetin, morin, quercetin, and rutin were determined by HPLC. The GBWE sample was filtered through a 0.45 μm filter (type Millex HV13, Millipore Corp., Bedford, MA, USA) prior to HPLC analysis, and 20 μL of the sample was injected into the HPLC equipment using a Merck-Hitachi L-6200 pump (Merck-Hitachi, Darmstadt, Germany), a UV-Visible detector Merck-Hitachi L-6200 pump (Merck-Hitachi, Darmstadt, Germany), and a reversed-phase LiChroCART RP-18 column (Merck, Darmstadt, Germany) (25 × 0.4 cm, particle size of 5 μm). The mobile phase consisted of 100% acetonitrile (solvent *A*) and 5% acetic acid solution (solvent *B*), and the following gradient elution programme was used: 0 min, 13% *A*/87% *B*; 25 min, 24% *A*/76% *B*; 40 min, 18% *A*/72% *B*; and 50 min, 13% *A*/87% *B*. The flow rate was maintained constant throughout the analysis at 1 mL/min. HPLC chromatograms of plant pigment (including anthocyanidin, myricetin, morin, rutin, and quercetin, respectively) standards and the GBWE are shown in Supplementary Figure 1.

2.3. Analysis of In Vitro Antioxidant Ability. The analyses of the DPPH radical-scavenging ability and reducing power were performed as described by Shimada et al. [18]. For the determination of the DPPH radical-scavenging activity, 1.5 mL of 0.15 mM DPPH in 50% ethanol was added to 1.5 mL of the sample solution with varying GBWE concentrations (0.5, 1, 2, and 4 mg/mL). The mixture was mixed vigorously and incubated at room temperature in the dark for 30 min. The optical density at 517 nm was measured using a microplate reader (BioTek Instruments Inc.). Vitamin C (1 mg/mL) was used as a control. The scavenging activity was calculated as $(1\text{-}A_{GBWE}$ or $A_{\text{vitamin C}}/A_{blank}) \times 100$. The data are presented as percentages relative to the control (vitamin C).

To determine the reducing power, 0.5 mL of the sample solution (0.05, 0.1, 0.25, 0.5, 1, and 2 mg/mL GBWE) was mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide, and the mixture was incubated at 50°C for 20 min. An aliquot (2.5 mL) of 10% trichloroacetic acid was added to the mixture, and the mixture was then centrifuged at 3000 × g for 10 min. The upper layer of solution (2.5 mL) was mixed with 2.5 mL of distilled water and 2.5 mL of 0.1% ferric chloride, and the absorbance at 700 nm was read using a microplate reader (BioTek Instruments Inc.). Vitamin C (1 mg/mL) was used as a control. The reducing power was calculated as $(A_{GBWE}$ or $A_{\text{vitamin C}} - A_{blank})/A_{\text{vitamin C}} \times 100$. The data are presented as percentages relative to the control (vitamin C).

The chelation of ferrous ions by 0.5, 1, 2, or 4 mg/mL GBWE was estimated as described by Dinis et al. [19], and Na_2EDTA (10 mM) was used as the positive control. The iron chelators (940 μL) at working concentrations of 1 and 2 mM were each mixed with 20 μL of ferrous sulphate (2 mM), and ferrozine was added to a concentration of 0.2 mM to start the reaction. The resulting mixture was mixed thoroughly and incubated for 10 min at room temperature. The absorbance of the solution at 562 nm was measured using a microplate reader (BioTek Instruments Inc.). The chelating activity was calculated as $(A_{EDTA}\text{-}A_{GBWE}/A_{EDTA}) \times 100$. The data are presented as percentages relative to the control (EDTA).

The superoxide radical-scavenging ability was measured using a commercially available RANSOD kit (cat. no. SD125; Randox Laboratories Ltd.) according to the manufacturer's recommended protocol, and SOD was used as the positive control.

2.4. Animals and Treatments. Thirty 4-week-old male Syrian hamsters were purchased from the National Laboratory Animal Center. All animal care and experimental protocols used in the present study were approved by the Institutional Animal Care and Use Committees at Fooyin University (Kaohsiung, Taiwan). The hamsters were treated in compliance with the Guide for the Care and Use of Laboratory Animals [20]. This study was initiated (design of the study and acquisition of funding), performed, and finished in 2005–2006. After 1 week of acclimation, the hamsters were randomly divided by weight into five groups of six hamsters each and maintained under a 12 h light/12 h dark cycle with free access to water and an AIN-93G-based diet (5% soybean oil; ND) or an HFD (20% soybean oil plus 0.5% cholesterol) (Dyets, Inc.). Hamsters fed the HFD were subdivided into different treatment groups: the hamsters belonging to the GBWE-L and GBWE-H groups were orally administered 0.4 and 0.8 g/kg body weight (BW) GBWE, respectively; and the hamsters belonging to the probucol group were administered 30 mg/kg BW probucol (Sigma-Aldrich; Merck KGaA), which is a drug that is used for lowering LDL cholesterol in clinical settings and was used as a positive control in this study, based on the method developed by Moghadasian et al. [21], reference to the excellent antioxidative ability of probucol after supplement 3 times per week for 2 weeks [22]. The GBWE dose used in the present study was determined based on our previous studies [7, 8]. The dosing frequency in the present study was referred to as supplemental food and functional food studies model [23, 24]. All GBWE products and probucol were dissolved in soybean oil and administered orally four times per week for 12 weeks. At the end of the treatment, the hamsters were fasted overnight and sacrificed by carbon dioxide euthanasia. To decrease the pain from CO_2-based euthanasia, the displacement rate used was between 20 and 30% of the chamber volume per minute. Death of the hamsters was confirmed by the lack of a pulse, breathing, corneal reflex, and response to a firm toe pinch and an inability to hear respiratory sounds and the heartbeat using a stethoscope. During the experimental period, body weight and food intake were recorded every other day. The food efficiency ratio (FER) was calculated by dividing the total weight gain (g) by diet consumption (g).

The whole blood was collected, incubated at room temperature for 30 min, and subsequently centrifuged at 1,100 × g and 4°C for 10 min. The upper fraction (serum) was collected and prepared for lipid profile analysis. The liver, heart, spleen, and kidney were removed and weighed. After removal, the fresh livers were minced, rapidly freeze-clamped in liquid nitrogen, and stored at −80°C.

2.5. Histological Examination. The liver tissues were fixed in 10% neutral-buffered formalin for 6 h at room temperature, and the formalin was then replaced with fresh formalin. Subsequently, the tissues were incubated overnight at room temperature, and 5 μm thick sections obtained from the paraffin-embedded tissues were stained with haematoxylin and eosin (H&E) at room temperature using the Harris haematoxylin and eosin staining protocol. The sections were then dehydrated using ascending alcohol solutions (50, 70, 80, 95, and 100%) at room temperature, cleared three to four times with xylene, and mounted on glass slides using Permount (Thermo Fisher Scientific, Inc.). Pathological examinations were performed by a blinded expert pathologist.

2.6. Serum Lipid Determination. The whole blood was collected, incubated at room temperature for 30 min, and subsequently centrifuged at 1,100 × g and 4°C for 10 min. The upper fraction (serum) was collected and prepared for lipid profile analysis. The levels of TC, TG, LDL-C, and high-density lipoprotein-cholesterol (HDL-C) were measured using commercial kits (cat. nos. CH202, TR212, CH2657, and CH2655, respectively; Randox Laboratories Ltd., San Diego, CA, USA) according to the manufacturer's recommended protocols.

2.7. Determination of Hepatic GSH and Oxidative Glutathione (GSSG) Levels. Liver tissue homogenates were prepared in four volumes of 10 mmol/L potassium phosphate, 150 mmol/L potassium chloride, and 1 mM PMSF, pH 7.4, and centrifuged at 10,000 × g and 4°C for 30 min. The supernatant of each group was combined with 1 mL of a solution with 10% perchloric acid and 2 mmol/L 1,10-phenanthroline. The levels of acid-soluble GSH and GSSG in the liver supernatants were measured by high-performance liquid chromatography as described previously [25].

2.8. Lipid Peroxidation Analysis. The lipid peroxidation of liver tissue was evaluated by measuring the formation of thiobarbituric acid reactive substances (TBARSs). Liver tissue homogenates were prepared in four volumes of 10 mmol/L potassium phosphate and 150 mmol/L potassium chloride, pH 7.4, and centrifuged at 10,000 × g and 4°C for 30 min. The hepatic TBARS levels were assayed as described by Fraga et al. [26]. Briefly, 0.2 mL of 3% sodium dodecyl sulphate and 0.05 mL of 4% BHT (in ethanol) were combined with 100 μL of liver homogenates. After mixing, 2 mL of 0.1 N HCl, 0.3 mL of 10% phosphotungstic acid, and 1 mL of 0.7% 2-thiobarbituric acid were added to the mixture. The resulting mixture was heated for 30 min in boiling water, and TBARSs were extracted into 5 mL of n-butanol. After centrifugation, the fluorescence of the butanol layer was measured with a Hitachi F-4500 fluorescence spectrophotometer using excitation and emission wavelengths of 515 nm and 555 nm, respectively. The homogenate protein concentrations in liver tissues were determined as described by Lowry et al. [27].

2.9. Analysis of Enzyme Levels Associated with Lipid Metabolism in the Blood. Liver tissue homogenates were prepared in four volumes of 10 mmol/l potassium phosphate, 150 mmol/L potassium chloride, and 1 mM PMSF, pH 7.4, and centrifuged at 10,000 × g and 4°C for 30 min. The protein concentrations of the liver tissue homogenates were determined as described by Lowry et al. [27]. Subsequently, 10–20 μg of total protein from each hepatic sample was loaded on a 10% SDS gel, resolved by 10% SDS-PAGE [28], and transferred to PVDF membranes [29]. The membranes were incubated with anti-HMG-CoA reductase (1 : 1,000 dilution; cat. no. ab174830, Abcam), anti-ACAT-2 (1 : 500 dilution; cat. no. ab168342, Abcam), or anti-LCAT (1 : 500 dilution; cat. no. ab109417, Abcam) antibodies at 37°C for 1 h and then with a peroxidase-conjugated anti-rabbit immunoglobulin *G* secondary antibody (1 : 10,000 dilution; cat. no. ab6721; Abcam) at 37°C for 1 h. The signals were visualized using an enhanced chemiluminescence detection kit (GE Healthcare). For densitometry analysis, the blots were treated with enhanced chemiluminescence reagent and exposed using a ChemiDoc XRS + system (Bio-Rad Laboratories, Inc.).

2.10. Statistical Analysis. The data were analyzed using SPSS version 20.0 (IBM Corp.). The differences between groups or positive groups were compared by one-way ANOVA with a post hoc Duncan's, respectively. The data are presented as the means ± standard deviations, and $p < 0.05$ was considered to indicate a statistically significant difference.

3. Results

3.1. Plant Pigment Contents in the GBWE. The primary polyphenolic compounds in the GBWE were 0.24 μg/g anthocyanin, 0.001 μg/g myricetin, and 0.175 ng/g morin, but no quercetin or rutin was detected in the GBWE in the present study (data not shown). The HPLC chromatograms of the plant pigment standards and the GBWE are shown in Supplementary Figure 1.

3.2. In Vitro Antioxidant Activity of the GBWE. In the present study, the antioxidant effects of the GBWE were evaluated by assessing its ability to quench free radicals (reducing power). The DPPH-mediated reduction of free radicals obtained with 0.5, 1, 2, and 4 mg/mL GBWE was 34.52 ± 2.0%, 43.94 ± 1.8%, 50.30 ± 1.4%, and 56.03 ± 1.2% of that obtained with vitamin C (100%), respectively (Figure 1(a)). As shown in Figure 1(b), the ferrous ion-chelating ability obtained with 0.5, 1, 2, and 4 mg/mL GBWE was 54.89 ± 10.82%, 64.53 ± 12.71%, 78.47 ± 6.32%, and 81.30 ± 10.32% of that found with EDTA (100%), respectively. The reducing power was 1.1–39.1% of that obtained with vitamin C (100%) (Figure 1(c)), and the superoxide radical-scavenging ability of the GBWE was 91–94% of that of the SOD standard (Figure 1(d)).

These results showed that the EC50 values of the GBWE based on its DPPH radical-scavenging ability, reducing power, and ferrous ion-chelation ability were 3.28, 1.94, and 0.37 mg/mL, respectively.

3.3. The GBWE Improves Fatty Liver in HFD-Fed Hamsters. After 12 weeks of feeding, no changes in the body weight gain or average food intake were found among the ND, HFD, GBWE-L, GBWE-H, and probucol groups (data not shown). However, as shown in Supplementary Table 1, the FER of the HFD group (0.08%) was significantly lower than that of the other groups (0.10–0.11%), which might explain why the body weight gain was not significantly increased in the HFD group. This finding shows that the HFD group exhibited a higher food conversion efficiency than the other groups.

The HFD caused a significant increase in liver weight in the HFD group ($p < 0.05$) but did not induce significant changes in the weight of the heart, kidney, or spleen (Table 1). Hamsters treated with GBWE-L, GBWE-H, or probucol exhibited a significant decrease in liver weight compared with those in the HFD group ($p < 0.05$), which suggested that the administration of GBWE-L, GBWE-H, or probucol improved the liver weight under HFD conditions. In addition, pathological tissue examinations of the livers from hamsters belonging to each group showed that fatty deposits (dotted arrows) were notably decreased in the animals administered GBWE-L, GBWE-H, or probucol, as shown by H&E staining (Figure 2). Furthermore, no significant changes in serum AST or ALT activity were found in the animals administered GBWE-L or GBWE-H, which suggested that the dose of the GBWE used did not result in liver toxicity (data not shown).

3.4. The GBWE Increases the Hepatic GSH Levels in HFD-Fed Hamsters. Hamsters that were administered GBWE-L, GBWE-H, or probucol for 12 weeks exhibited significantly increased hepatic GSH levels (49.4 ± 3.2, 56.7 ± 2.8, and 48.2 ± 2.4 nmol/mg protein, respectively) compared with those in the HFD group (43.6 ± 1.6 nmol/mg protein) ($p < 0.05$; Table 2). The GSSG contents of the hamsters belonging to the GBWE-L and GBWE-H groups (1.1 ± 0.1 and 1.0 ± 0.1 nmol/mg protein, respectively) were significantly lower than those found in the hamsters belonging to the HFD control group (1.6 ± 0.3 mg/mg protein) ($p < 0.05$; Table 2). The GSH/GSSG ratios obtained for the GBWE-L, GBWE-H, and probucol groups were significantly higher than those found for the HFD control group ($p < 0.05$). The TBARS levels in the hamsters belonging to the GBWE-L and GBWE-H groups were significantly lower than those in the HFD group (Table 2). These results show that the GBWE can increase the antioxidative ability and decrease oxidative stress in hamsters fed an HFD for 12 weeks.

3.5. The GBWE Reduces the Serum TC and LDL-C Levels. At the end of the 12-week feeding period, the TC levels of the hamsters in the HFD group (226 ± 16 mg/dL) were significantly higher (~182% higher) than those of the hamsters in the control group (124 ± 10 mg/dL; $p < 0.05$; Table 3).

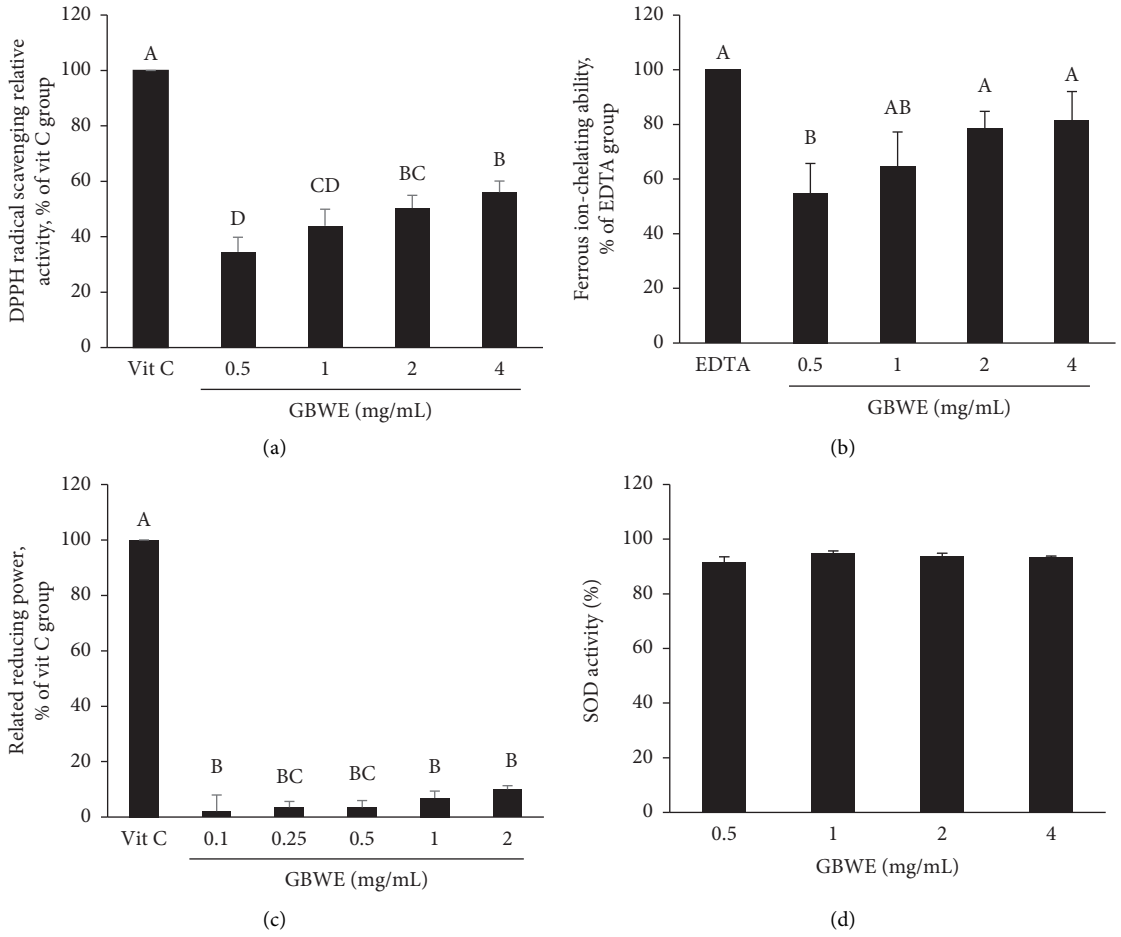

Figure 1: In vitro antioxidative ability of the GBWE. (a) DPPH radical-scavenging activity, (b) ferrous ion-chelating ability, (c) reducing power, and (d) superoxide radical-scavenging activity. Vitamin C was used as the positive control in the analyses of the DPPH radical-scavenging ability and reducing power, and Na_2EDTA was used as the positive control in the analysis of the chelation of ferrous ions. SOD was used as the positive control in the superoxide radical-scavenging ability assay. The data are expressed as the means ± standard deviations from three independent repeats. Groups with different letters (A, B, and C) are significantly different, as determined by Duncan's test ($p < 0.05$). GBWE, *G. bicolor* water extract.

Table 1: Effects of the GBWE on the relative organ weights of hamsters fed an HFD for 12 weeks.

Group	Liver	Spleen	Kidney	Heart
	%			
ND	3.68 ± 0.43^{c}	0.09 ± 0.01	0.74 ± 0.04	0.41 ± 0.02
HFD	8.29 ± 0.50^{a}	0.08 ± 0.02	0.67 ± 0.08	0.42 ± 0.06
Probucol	6.59 ± 0.41^{b}	0.08 ± 0.01	0.68 ± 0.05	0.39 ± 0.07
GBWE-L	6.22 ± 0.38^{b}	0.09 ± 0.01	0.70 ± 0.04	0.40 ± 0.06
GBWE-H	6.35 ± 1.07^{b}	0.09 ± 0.03	0.69 ± 0.02	0.36 ± 0.07

GBWE, *G. bicolor* water extract; HFD, high-fat diet; GBWE-L, 0.4 g/kg body weight GBWE; GBWE-H, 0.8 g/kg body weight GBWE. The values are presented as the means ± standard deviations from four to five repeats. Groups with different letters (a, b, and c) in the same column are significantly different, as demonstrated by Duncan's test ($p < 0.05$).

Probucol, GBWE-L, and GBWE-H significantly reduced the TC levels compared with those obtained in the HFD group ($p < 0.05$). The LDL-C levels of the GBWE-H group (56.3 ± 5.3 mg/dL) were significantly higher than those of the HFD control group (73.8 ± 9.6 mg/dL) ($p < 0.05$). Additionally, the GBWE-H group exhibited significantly higher HDL-C levels than the other control groups, including the HFD and ND groups ($p < 0.05$). These results showed that GBWE can regulate the blood lipid profiles and lower the risk of high blood cholesterol under HFD conditions.

3.6. The GBWE Regulates the Levels of Hepatic Cholesterol Metabolism-Related Enzymes in HFD-Fed Hamsters. The effects of the GBWE on hepatic cholesterol metabolism-related enzymes in hamsters fed an HFD for 12 weeks are shown in Figure 3(a). The GBWE-L- and GBWE-H-treated groups ($96 \pm 9\%$ and $136 \pm 7\%$, respectively) exhibited significantly increased levels of hepatic LCAT compared with the HFD group ($68 \pm 8\%$) ($p < 0.05$) (Figure 3(b)), and the expression of LCAT in the ND group was considered equal to 100%. However, the GBWE did not affect the expression of HMG Co-A reductase and ACAT-2 in hamsters fed the HFD for 12 weeks (Figures 3(a) and 3(b)).

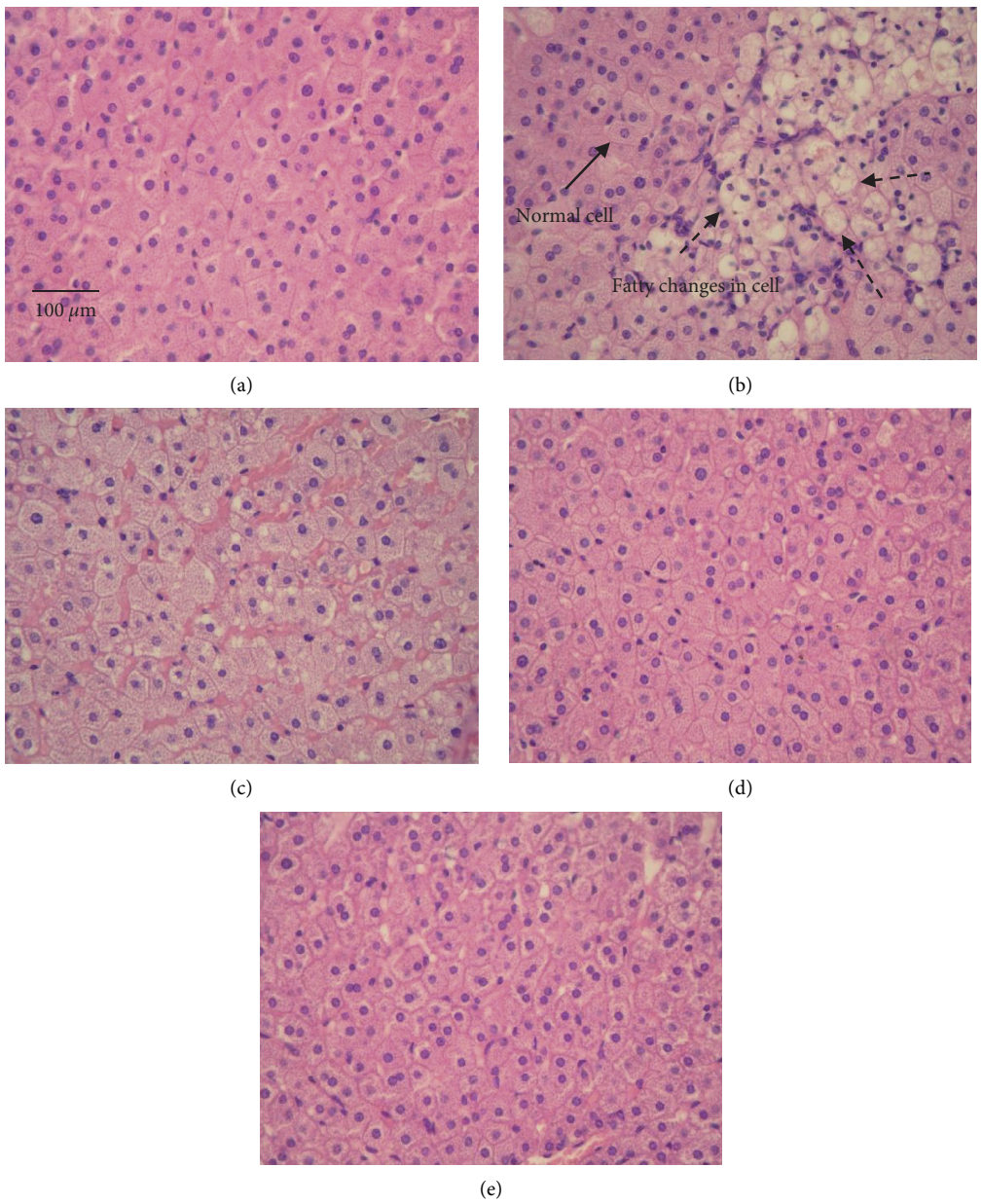

Figure 2: Effects of the GBWE on hepatic tissue of Syrian hamsters fed an HFD. The fatty changes, which represent steatosis, are indicated by arrows. GBWE, *G. bicolor* water extract; ND, normal diet; HFD, high-fat diet; GBWE-L, 0.4 g/kg body weight GBWE; GBWE-H, 0.8 g/kg body weight GBWE. The magnification in the images is 100x. (a) ND. (b) HFD. (c) Probucal. (d) GBWE-L. (e) GBWE-H.

4. Discussion

The present study demonstrated that the GBWE exhibits antioxidant ability in vitro and in vivo. Specifically, the GBWE attenuated the dysregulation of the blood lipid and lipoprotein profiles of hamsters fed an HFD by reducing oxidative stress and regulating cholesterol metabolism. The GBWE not only exhibited DPPH radical-scavenging ability and lowered ferrous ion-chelating ability in the in vitro model but also reduced HFD-induced oxidative stress by increasing the glutathione levels, as demonstrated in the in vivo model. In addition, the GBWE regulated the blood lipid levels, lipoprotein profiles, and LCAT expression. Notably, the GBWE reduced oxidative stress induced by an HFD, and these results highlight a novel method for potentially reducing hyperlipidaemia and cardiovascular disease risk in HFD-fed patients. The present study also showed that the GBWE regulated the blood lipid levels and lipoprotein profiles.

People are interested in both the colourful appearance and the healthful claim of *G. bicolor*. In the present study, the GBWE is our test material because people usually cook and eat *G. bicolor* through boiling, and thus, these research results can potentially be applied to our daily life. Our previous study showed that the GBWE contains chlorophyll, gallic acid, and β-carotene [8], which belong to three of four

Table 2: Effects of the GBWE on the TBARS and GSH levels in hamsters fed an HFD for 12 weeks.

Group	TBARS	GSH	GSSG	GSH/GSSG
	nmol/mg protein			%
ND	0.20 ± 0.02^{ab}	50.1 ± 1.4^{a}	1.2 ± 0.2^{b}	40.6 ± 2.0^{a}
HFD	0.28 ± 0.08^{a}	43.6 ± 1.6^{b}	1.6 ± 0.3^{a}	26.7 ± 1.6^{b}
Probucol	0.21 ± 0.05^{ab}	48.2 ± 2.4^{a}	1.2 ± 0.1^{b}	39.3 ± 3.0^{a}
GBWE-L	0.16 ± 0.03^{b}	49.4 ± 2.2^{a}	1.1 ± 0.1^{b}	44.1 ± 2.7^{a}
GBWE-H	0.19 ± 0.04^{b}	56.7 ± 2.8^{a}	1.0 ± 0.1^{b}	56.2 ± 3.1^{a}

TBARSs, thiobarbituric acid reactive substances; GSH, glutathione; GSSG, oxidative glutathione; GBWE, *G. bicolor* water extract; HFD, high-fat diet; GBWE-L, 0.4 g/kg body weight GBWE; GBWE-H, 0.8 g/kg body weight GBWE. The values are presented as the means ± standard deviations from four to five repeats. Groups with different letters (a, b, and c) in the same column are significantly different, as demonstrated by Duncan's test ($p < 0.05$).

Table 3: Effects of the GBWE on the blood lipid and lipoprotein profiles of hamsters fed an HFD for 12 weeks.

Group	TGs	TC	LDL-C	HDL-C
	mg/dL			
ND	27.4 ± 5.1	124 ± 10^{c}	36.6 ± 5.9^{c}	98 ± 8^{c}
HFD	32.6 ± 4.8	226 ± 16^{a}	73.8 ± 9.6^{a}	165 ± 15^{b}
Probucol	30.0 ± 6.5	165 ± 13^{b}	68.3 ± 4.2^{ab}	164 ± 12^{b}
GBWE-L	28.2 ± 8.3	171 ± 15^{b}	68.3 ± 10.3^{ab}	162 ± 12^{b}
GBWE-H	28.8 ± 7.1	159 ± 11^{b}	56.3 ± 5.3^{b}	186 ± 10^{a}

TC, total cholesterol; TG, total triglyceride; LDL-C, low-density lipoprotein-cholesterol; HDL-C, high-density lipoprotein-cholesterol; GBWE, *G. bicolor* water extract; HFD, high-fat diet; GBWE-L, 0.4 g/kg body weight GBWE; GBWE-H, 0.8 g/kg body weight GBWE. The values are presented as the means ± standard deviations from four to five repeats. Groups with different letters (a, b, and c) in the same column are significantly different, as demonstrated by Duncan's test ($p < 0.05$).

major plant pigment groups, namely, the chlorophyll, flavonoid, and carotenoid groups [13, 14]. Some other flavonoids, including anthocyanidin, myricetin, and morin, were identified as components of the GBWE in the present study, and these components of the GBWE provide *G. bicolor* plants with their characteristic pigmentation and might also underlie their beneficial physiological effects [1]. To understand the major plant pigments of the GBWE and their effect on blood and lipoproteins, the three types of plant pigments were analyzed and discussed in this study. Our previous study prepared a *G. bicolor* water extract (GBWE), *G. bicolor* alcohol extract (GBAE), and *G. bicolor* ether extract (GBEE) as the experimental materials [8], and in the present study, we not only analyzed the plant pigment composition of the GBWE but also determined their antioxidation effects in vitro. Our previous study showed that the GBWE contains a few pigment compounds (22.5 μg/g chlorophyll and 1.63 μg/g β-carotene) and undetectable levels of quercetin and rutin, but the ethanol and ether extracts of *G. bicolor* contain different amounts of these compounds. For example, the GBEE contains 25.3 μg/g quercetin, 11500 μg/g chlorophyll, and 7460 μg/g beta-carotene [8]. Although these pigment compounds were found in small amounts, other studies have shown that low concentrations of these compounds exert various physiological effects. For example, approximately 21 mg/100 g (210 μg/g) chlorophyll and 1.5 mg/100 g (15 μg/g) β-carotene are found in the extracts of wild watercress (*Nasturtium officinale* L.); although present in small amounts, these compounds play an important role in antioxidation [30]. The extracts of cranberry contain 1.78 g/100 g myricetin (17.8 μg/g) and undetectable amounts of rutin and gallic acid but still exhibit excellent potential for protection against colonic cancer [31]. The results from these previous studies as well as the present study show that the physiological effects of these plant extracts might be obtained from the whole extracts or the potential existence of synergistic or additive effects between various components of the GBWE.

Epidemiological studies have shown that an increased intake of fruits and vegetables increases the concentration of antioxidants, such as vitamin C, lutein, and carotenoids, in the blood [32]. Zern and Fernandez [33] also found that dietary polyphenols, which are commonly found in vegetables and fruits, decrease the effects of cardiovascular disease. The administration of an HFD containing 10% corn oil and 0.2% cholesterol with tomato paste (containing 0.11% lycopene) to Syrian hamsters for 8 weeks decreased their serum TC and LDL-C levels by 14.3% and 11.3%, respectively, compared with those found in the hamsters fed an HFD alone [34]. Among the analyses of various *Gynura* family plants, the *Gynura procumbens* water extract lowered the TC, TG, and LDL-C levels effects in Sprague-Dawley (SD) rats fed an HFD to induce chronic hyperlipidaemia [35]. Ahmad Nazri et al. [36] showed that the *Gynura procumbens* ethanol extract can reduce the TG, TC, and LDL-C levels in SD rats fed a 2% cholesterol diet. In the present study, Syrian hamsters were fed an HFD, and the administration of GBWE-H lowered the serum TC and LDL-C levels by 29.6% and 23.3%, respectively. Together, the results of the present and previous studies demonstrate that fruits/vegetables and certain teas might reduce the incidence of arteriosclerosis. The mechanism through which GBAE regulates the TC, LDL-C, and HDL-C levels remains to be determined.

Of interest, the TG level of the HFD group was slightly increased compared with that of the ND group and the other experimental groups. Although it is an unusual phenomenon, in animal models, the fat percentage, cholesterol amount, and/or experimental time periods affect the induction of hyperlipidaemia [37]. In the present study, however, the levels of TC, LDL-C, and HDL-C in the HFD group were significantly higher than those in the ND group, which indicates that the regulatory effects of the HFD still exert a physiological effect on blood lipids and the lipoprotein profile. The HFD group had a higher HDL-C level than the ND group, which is also of particular interest in this study. However, some studies have shown that an HFD also enhances the increase in HDL-C, and the related mechanism is thought to be involved in reverse cholesterol transport [38]. The dietary fat-induced increase in the HDL-C levels might be an adaptive mechanism. This phenomenon is regulated by increasing the transport rate and LCAT activity and decreasing the fractional catabolic rates of HDL cholesterol ester and apolipoprotein A-1 [39]. In this study,

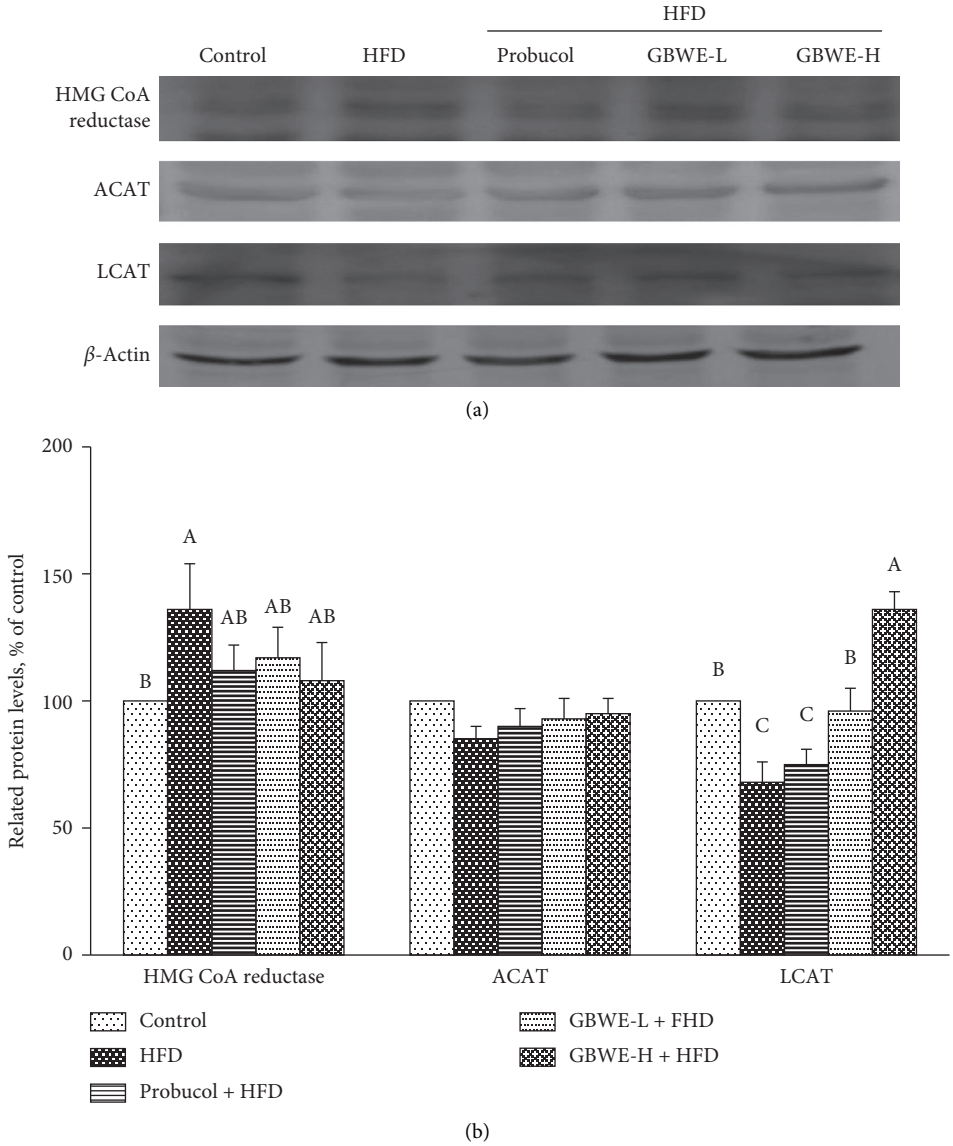

Figure 3: Effects of the GBWE on HMG-CoA reductase, ACAT, and LCAT protein levels in Syrian hamsters fed an HFD. The values are presented as the means ± standard deviations from four to five repeats. Groups with different letters (A, B, and C) in the same column are significantly different, as demonstrated by Duncan's test ($p < 0.05$). GBWE, *G. bicolor* water extract; ND, normal diet; HFD, high-fat diet; GBWE-L, 0.4 g/kg body weight GBWE; GBWE-H, 0.8 g/kg body weight GBWE; HMG-CoA reductase, 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase; LCAT, lectin-cholesterol acetyltransferase; ACAT, acyl-coenzyme A:cholesterol acyltransferase.

hamsters were fed an HFD containing 20% fat and 0.5% cholesterol, and the induction of the HDL-C levels might have occurred through one of the abovementioned mechanisms. In addition, the probucol group exhibited slightly decreased TG and LDL-C levels compared with the HFD group. We hypothesize that the regulatory mechanism, dosage, and frequency of probucol will affect the low LDL-C levels. In addition, the main mechanism through which probucol regulates atherosclerosis involves the reduction of LDL-C formation and not the TG levels [40].

The antioxidants in fruits and vegetables have garnered increasing attention due to their physiological benefits and have been demonstrated to exhibit various favourable properties, particularly the reduction of arteriosclerosis [41]. Among the phytochemicals found in the GBWE, anthocyanins reduce oxidative damage in the liver [42], and carotenoids exhibit a free radical-scavenging ability and beneficial antioxidant activity [43]. Krishnan et al. [44] showed that the *Gynura procumbens* water extract has the ability to scavenge DPPH radicals and reduce the antioxidant power of ferric ions in vitro. Ahmad Nazri et al. [36] also reported that the *Gynura procumbens* ethanol extract can reduce the MDA levels in SD rats fed an HFD for 24 weeks. In the present study, the GBWE exhibited antioxidant activity due to its ability to scavenge free radicals, decrease the lipid peroxide levels, and increase the hepatic GSH levels. However, the TBARS levels of the HFD group were slightly higher than those of the ND group, which might be related to

the composition of the experimental HFD. The diet used in this study had a relatively lower fat content (20% of total calories of soybean oil) and/or a relatively lower saturated fat level compared with the HFDs used by Echeverría et al. [45] (containing 60% of total calories), Karmakar et al. [46] (containing 28% of total calories), and Dorfman et al. [47] (containing 62.5% of total calories). All the abovementioned studies successfully induced oxidative stress and hyperlipidaemia; thus, both the percentage of fat among the total calories and the saturated fat levels play important roles in the induction of hyperlipidaemia and oxidative stress. However, the TBARS levels of the GBWE-L and GBWE-H groups were significantly lower than those of the HFD group ($p < 0.05$). These results showed that the GBWE exerts an antioxidation effect in hamsters fed an HFD. In our previous study, it was found that the GBWE also reduced the nitrogen oxide and PGE2 levels, which are inflammatory markers, by inhibiting NF-κB activation [7]. Therefore, the beneficial properties of the phytochemicals in the GBWE might be due to their antioxidative and anti-inflammatory responses in vivo and in vitro.

The GBWE also reduces fatty changes to hepatocytes, which is a sign of steatohepatitis stress (Figure 2), and improves hepatic function, as shown by measuring the serum AST and ALT levels, and these effects might underlie the observed reductions in oxidative and inflammatory stress. The long-term administration of an HFD to animals will result in increased oxidative stress and dysfunctional mitochondria in several organs [48]. In obese patients, the excess deposition of TGs in hepatocytes followed by the development of inflammatory nonalcoholic steatohepatitis (NASH) and fibrogenic responses results in progression to nonalcoholic fatty liver disease (NAFLD) [49]. Previous studies have shown that black tea extract can protect against HFD-induced NASH in Wistar rats by decreasing the TG, TC, and LDL-C levels and increasing the SOD and GSH levels [46]. Yin et al. [4] used alcohol to induce mouse liver damage and showed that the GBWE reduced the accumulation of hepatic lipids and oxidative and inflammatory injury in C57BL/6 mice fed a Lieber-DeCarli liquid diet with ethanol. In particular, the TG levels of the HFD group were not significantly higher than those of the ND group. However, the fatty changes in hepatocytes are quite visible in Figure 2. The degree of steatosis in both the GBWE-L and GBWE-H groups was markedly decreased. Fan et al. [50] showed that an increase in NAFLD is associated with increased TG, TC, and TG/HDH-C levels, decreased HDL-C levels, and metabolic syndrome. The mechanism through which GBWE reduces steatosis needs future study.

In the present study, the GBWE increased the LCAT protein levels in hamsters fed an HFD. LCAT is an enzyme that converts free cholesterol into cholesteryl ester and promotes newly synthesized HDL to form a more complete sphere. LCAT lowers serum free cholesterol and enhances HDL-C production [51]. In a clinical trial, patients with hypercholesterolaemia were administered β-sitosterol, and their LCAT expression levels were significantly increased [52]. The GBWE might be involved in increasing LCAT expression and thus the regulation of cholesterol metabolism. Despite this novel finding, the mechanism needs further study.

5. Conclusion

The present study showed that the GBWE exhibits antioxidant activity, regulates the blood lipid and lipoprotein levels in hamsters fed an HFD, and controls the expression of LCAT, which is involved in the related metabolic pathways.

Data Availability

The datasets used and/or analyzed during the present study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Supplementary Materials

Supplementary Figure 1: high-performance liquid chromatography chromatograms of (A) the anthocyanidin, myricetin, morin, rutin, and quercetin standards and (B) the GBWE. (*Supplementary Materials*)

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