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# Mulberry Water Extracts Possess an Anti-obesity Effect and Ability To Inhibit Hepatic Lipogenesis and Promote Lipolysis

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**ABSTRACT:** Obesity plays a critical role in dyslipidemia and related disorders. Mulberry water extracts (MWEs) contain polyphenols, including gallic acid, chlorogenic acid, rutin, and anthocyanins. In this study, using 6-week-old male hamsters, we investigated the anti-obese effect of MWEs. After 12 weeks of treatment, MWEs lowered high-fat diet (HFD)-induced body weight and visceral fat, accompanied with hypolipidemic effects by reducing serum triacylglycerol, cholesterol, free fatty acid, and the low-density lipoprotein (LDL)/high-density lipoprotein (HDL) ratio ( $n = 8$  for each group). MWEs decreased hepatic lipids, thus protected livers from impairment. The hepatic peroxisome proliferator-activated receptor  $\alpha$  and carnitine palmitoyltransferase-1 were elevated, while fatty acid synthase and 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase were reduced by MWEs, indicating that MWEs regulated lipogenesis and lipolysis, which exerted the anti-obese and hypolipidemic effects. Noticeably, MWEs showed both efficacy and safety *in vivo*. In conclusion, MWEs can be used to reduce body weight, serum, and liver lipids.

**KEYWORDS:** Mulberry water extracts, anti-obese, hypolipidemic, lipogenesis, lipolysis

## INTRODUCTION

Obesity is associated with a great diversity of diseases, including metabolic syndrome and cardiovascular disease. In recent years, there is increased recognition that abdominal obesity and body fat deposition play a critical role in the pathogenesis of related disorders.<sup>1</sup>

There is a metabolic rationale linking the expanded abdominal or visceral fat depot to high triacylglycerol (TG), low high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) dyslipidemia, and insulin resistance, which are often accompanied with impaired metabolic regulation in adipose tissue, leading to an overproduction of free fatty acid (FFA).<sup>2</sup> Such flux of FFA toward the liver results in an increase of TG deposition and secretion of TG-rich lipoproteins, which in turn affect the lipolipase activity and the distribution of lipoprotein subtypes.

There was evidence showing that the hepatic lipase activity was elevated in visceraally obese patients.<sup>3</sup> Actually, the regulation of hepatic lipid metabolism, including both the lipogenesis and lipolysis, should be emphasized for preventing dyslipidemia and the accompanying illness. Expressions of fatty acid synthase (FAS) and 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the important enzymes regulating TG and cholesterol synthesis, were indicated as the markers of lipogenesis.<sup>4</sup> On the other hand, the expressions of carnitine palmitoyltransferase-1 (CPT-1) and peroxisome proliferator-activated receptor

$\alpha$  (PPAR $\alpha$ ) were critically associated with the process of lipolysis.

Mulberry, the fruit of *Morus alba* L., is traditionally used in Chinese medicines as a pharmaceutical for antifever diuretics, liver protection, blood pressure reduction, and cardiovascular disease prevention. Mulberry had been shown to contain water-soluble anthocyanins, a polyphenol exhibiting antioxidant and antitumor properties.<sup>5,6</sup> Actually, types of polyphenolic acid liberating from esters and glycosidic bonds have been reported in the composition of small berries.<sup>7</sup> Many herbal-derived polyphenolic compounds were suggested, capable of preventing obesity via hypolipidemia effects and adipose tissue reduction, and thus suppressed the occurrence of metabolic, hepatic, and cardiovascular alterations associated with obesity.<sup>8–12</sup>

Recently, it was demonstrated that the water extracts of mulberry fruit (MWEs) reduced serum levels of TG and total cholesterol and enhanced the gene expression of LDL receptor and LDL uptake in liver cells.<sup>13</sup> To further explore MWE effects, we use the high-fat diet (HFD)-fed hamster model, which resembles lipid distributions of human beings, to investigate whether MWEs possess the anti-obese effect and their influence

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**Table 1.** Retention Time, UV–Vis, and Mass Spectral Characteristics of the Compositions Present in MWEs

peak	compound	$R_t$ (min) <sup>a</sup>	UV $\lambda_{\max}$ (nm) <sup>b</sup>	$[M - H]^-$ <sup>c</sup>	MS <sup>2</sup>
1	gallic acid	5.0	272, 228	169	125
2	protocatechuic acid	10.53	262, 296, 230	153	109
3	3-caffeoyl quinic acid	14.6	231, 277, 285 sh, 317	353	191, 179, 135
4	chlorogenic acid	18.07	328, 298 sh, 244	353	191, 179, 135
5	4-caffeoyl quinic acid	19.01	330, 305 sh, 238	353	179, 173, 191, 135
6	caffeic acid	19.70	324, 296 sh, 244	179	135
7	rutin	24.79	348, 236, 299 sh, 255 sh	609	300, 301, 343
8	quercetin-3-O-glucoside	26.25	358, 357, 230 sh	463	301, 343, 300, 271, 179

<sup>a</sup>  $R_t$  (min) = retention time. <sup>b</sup> UV  $\lambda_{\max}$  (nm) = maximum absorption in the UV region. <sup>c</sup>  $[M - H]^-$  = molecular mass of mulberry phenolics on the loss of one proton measured by SIM.

on body weight, body fat content, serum lipid and lipoprotein levels, and the expression of enzymes regulating lipid metabolism.

## MATERIALS AND METHODS

**Preparation of MWEs.** MWEs were prepared from the fruit of *M. alba* L. (mulberry), which were obtained from the Taichung District Agricultural Research Station in TaiPin, Taichung, Taiwan. Briefly, the fruit (100 g) was macerated, stirred with water (200 mL), and centrifuged (3000 rpm, 15 min). The juice was filtered and concentrated under reduced pressure (about 30 cmHg) at 30 °C. Then, the aqueous extract was lyophilized (−80 °C, 12 h) to obtain MWE, which was stored at −20 °C before use.

**High-Performance Liquid Chromatography/Electron Spray Ionization–Tandem Mass Spectrometry (HPLC/ESI–MS–MS) Analysis.** HPLC with diode-array detection (DAD) was optimized and validated for the determination of mulberry polyphenolic compounds. HPLC was a Finnigan Syrrvey modulator HPLC system (Thermo Electron, Bedford, OH), equipped with an analytical column, Luna, 3.0  $\mu$ m, C18(2) column (2.00  $\times$  150 mm, Phenomenex, Inc., Torrance, CA) and a guard column. The spectral monitor was performed by a photodiode array (PDA) detector (Thermo Electron Co., Waltham, MA). The analyses were carried out using the mobile phase composed of two solvents: solvent A (water containing 0.1% formic acid) and solvent B (acetonitrile containing 0.1% formic acid). The flow rate was 0.2 mL/min. The elution was carried out in a programmed gradient elution as follows: 0–3 min, isocratic with 5% B; 3–15 min, with 5–20% B; 15–20 min, isocratic with 20% B; 20–30 min, with 20–30% B; 30–45 min, with 30–90% B; 45–50 min, isocratic with 90% B; and 50–60 min, from 90% B shifted to 5% B. The absorption spectra of eluted compounds were scanned within 190–600 nm using the in-line PDA detector monitored at 278, 360, and 518 nm.

The eluents were successively analyzed by ESI–MS–MS using an electron spray ionization tandem mass spectrometer (Finnigan LCQ Advantage MAX ion-trap mass spectrometer) operating in a negative mode. The extracts were filtered with 0.45  $\mu$ m Micropore. Aliquots of 20  $\mu$ L of the filtrate were directly injected into the column using a Rheodyne (model 7725i) injection valve. The operating conditions involved the following: spray needle voltage, 3.5 kV; capillary voltage, 16 V; tube lens offset, 55 V; ion transfer capillary temperature, 320 °C; nitrogen sheath gas, 45 units; and auxiliary gas, 5 units (in arbitrary units). The single-ion-monitoring (SIM) mode was used for quantifying the molecular ions of phenolic compounds. The SIM analysis was a narrow scan event monitored at the  $m/z$  value of the selected ion in the range of 1.0 Th, centered at the peak for the molecular ion. In this experiment, the SIM analysis was scanned in the  $m/z$  range of 150–800 with five microscans and a maximum ion injection time of 200 ms. In performing MS–MS analysis, helium collision gas was introduced in

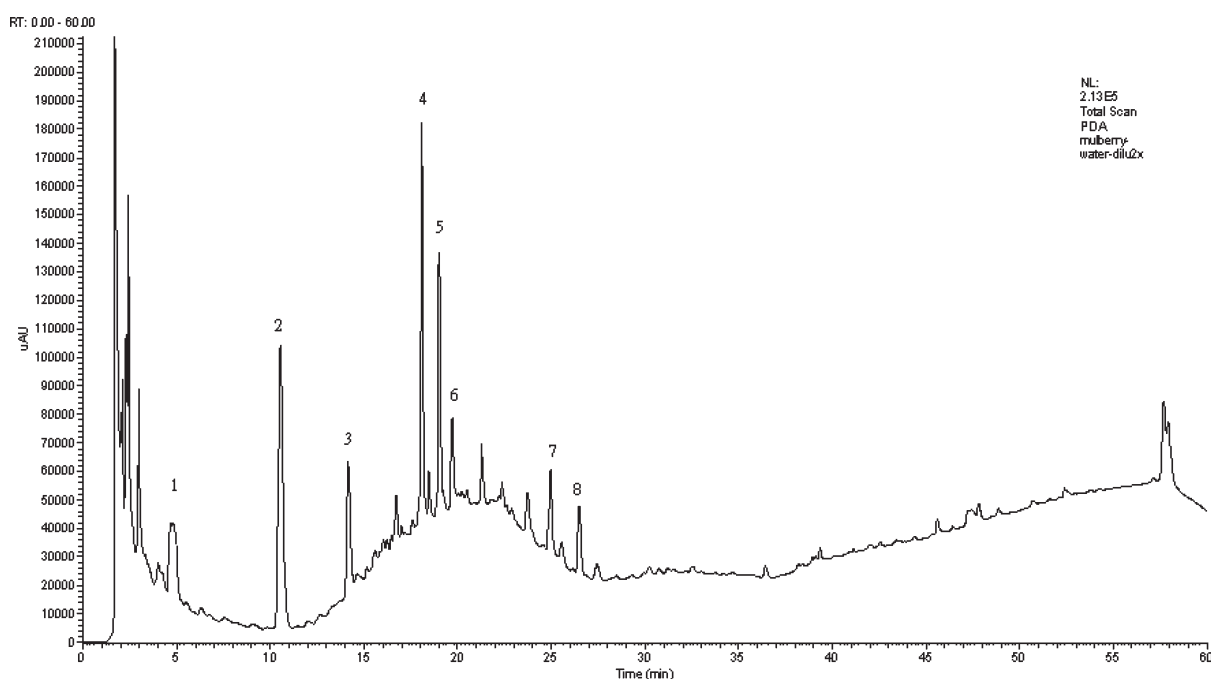
accordance with the recommendations of the manufacturer. The MS–MS fragment spectra was produced using normalized collision energy with an increment of 30% and also with wideband activation “off”.<sup>14</sup>

**Animal Experiments.** Male Syrian golden hamsters (6 weeks old) were purchased from the National Laboratory Animal Breeding and Research Center (Taipei, Taiwan). Hamsters were housed and acclimated in laboratory conditions (18–23 °C, 55–60% relative humidity, and 12 h light/dark cycle) for at least 1 week before each study. The chow (Laboratory Rodent Diet 5001, Lab Diet/PMI Nutrition International, Purina Mills LLC, Gray Summit, MO) was added with 0.2% cholesterol and 10% corn oil to obtain the HFD. Hamsters were fed with different diets and divided into the following groups ( $n = 8$  per group): (A) standard chow (control), (B) HFD, (C) HFD supplemented with 0.5% (w/w) MWEs (0.5% MWEs), (D) HFD supplemented with 1% (w/w) MWEs (1% MWEs), and (E) HFD supplemented with 2% (w/w) MWEs (2% MWEs). All of the hamsters were fed assigned diets and water *ad libitum* and were weighed every week. After 12 weeks, these animals were sacrificed by decapitation. Blood samples and liver tissue were collected, and serum was prepared and stored at −80 °C for further investigations. All animal experimental protocols used in this study were approved by the Institutional Animal Care and Use Committee of the Chung Shan Medical University (IACUC, CSMU), Taichung, Taiwan.

**Serum Biochemical Assays.** The serum sample was collected using ethylenediaminetetraacetic acid (EDTA) tubes and centrifuged at 3000 rpm for 10 min at 4 °C. Concentrations of TGs, total cholesterol, LDL cholesterol (LDL-C), HDL cholesterol (HDL-C), FFA, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and creatinine were measured by enzymatic colorimetric methods using commercial kits (Randox Laboratories, Ltd., Antrim, U.K.).

**Determinations of Total Cholesterol and TGs in Liver.** After removal from the animals, samples of fresh liver were collected for analyzing the contained lipid. Liver lipids were extracted with the following procedures. Briefly, liver (1.25 g) was homogenized with 10 mL of chloroform/methanol (v/v, 1:2) and then added and thoroughly mixed with chloroform (1.25 mL) and distilled water (1.25 mL). After centrifugation (1500g for 10 min), the lower clear organic phase solution was transferred into a new glass tube and then lyophilized. A total of 0.1 g of lyophilized powder was dissolved in 1 mL of chloroform/methanol (v/v, 1:2) as the liver lipid extract and stored at −20 °C for less than 3 days. The liver cholesterol and TGs in the lipid extracts were measured by enzymatic colorimetric methods using commercial kits (Randox Laboratories, Ltd., Antrim, U.K.).

**Preparation of the Protein Extract of Liver.** The liver chops were added with radioimmunoprecipitation assay (RIPA) buffer and protein inhibitors and homogenized at 4 °C. The tissue homogenates were centrifuged (10000g for 20 min at 4 °C), and the resulting supernatants (whole-tissue extracts) were used for the western blot analyses. The total protein concentrations of the whole tissue extracts were determined through the Bradford assay (Bio-Rad, Hercules, CA).



**Figure 1.** HPLC/UV chromatogram of MWEs showing eight major compounds. The eight compounds correspond to peaks 1–8, respectively. The peak numbers and their identifications were referenced in Table 1.

**Table 2.** Effect of MWEs on the HFD-Induced Body Weight Change<sup>a</sup>

group	weeks			
	0	2	8	10
control	95.6 ± 3.96	117.2 ± 5.06 (22.16)	122.0 ± 4.10 (27.7)	121.3 ± 6.75 <sup>b</sup> (26.7)
HFD	94.1 ± 8.23	124.2 ± 8.23 (32.0)	128.8 ± 5.55 (37.0)	130.9 ± 7.61 (39.1)
0.5% MWEs	93.3 ± 11.83	121.3 ± 10.84 (30.0)	124.6 ± 6.83 (33.5)	123.13 ± 4.43 (32)
1.0% MWEs	92.4 ± 7.01	116.7 ± 12.61 (26.3)	122.9 ± 16.4 (33.1)	121.6 ± 3.97 <sup>b</sup> (31.7)
2.0% MWEs	92.1 ± 9.90	117.09 ± 9.90 (27.2)	121.53 ± 6.58 (31.9)	120.41 ± 3.81 <sup>c</sup> (30.8)

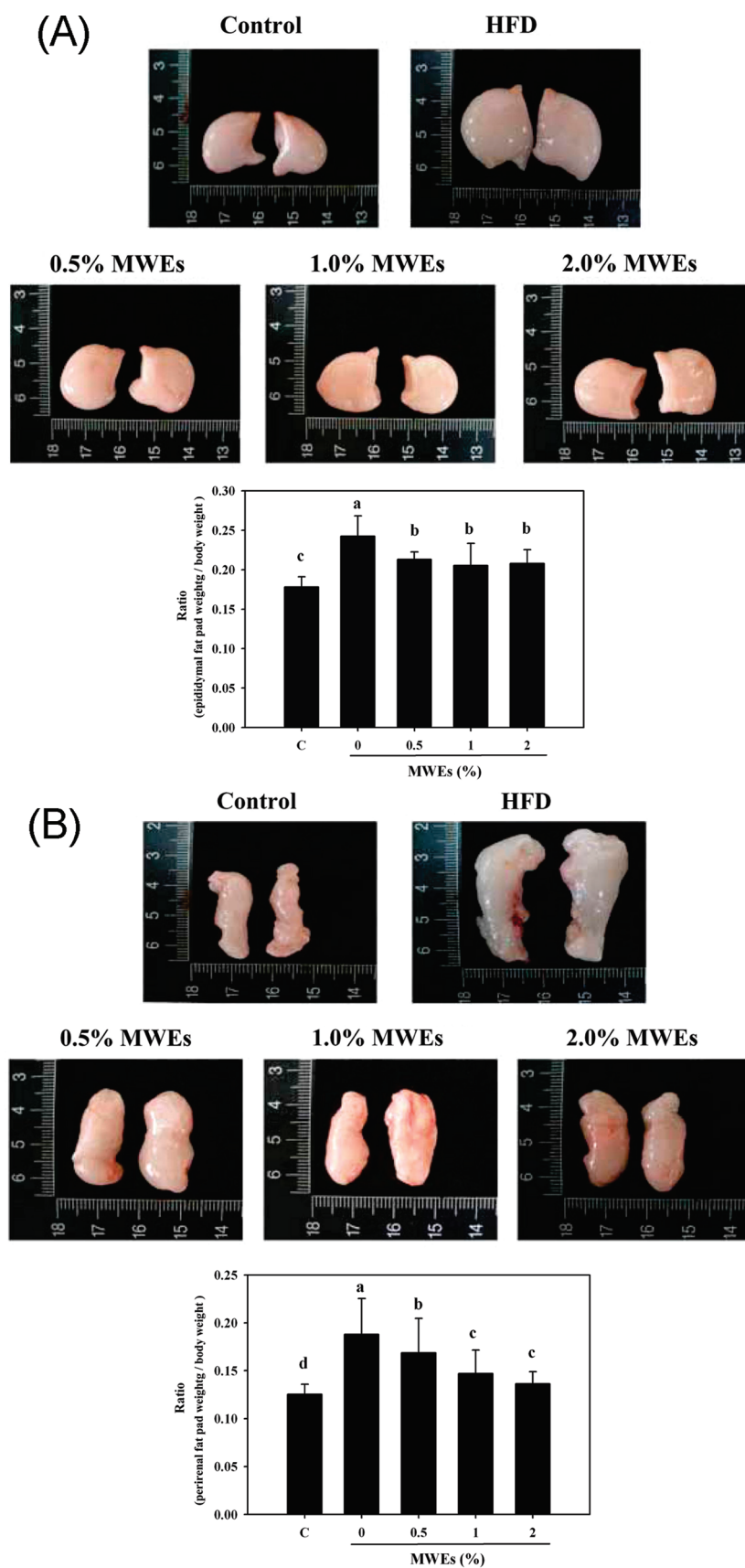
<sup>a</sup> Hamsters were fed with different diets and divided into the following groups: control, fed with standard chow; HFD, fed with chow added with 0.2% cholesterol and 10% corn oil; 0.5% MWEs, fed with HFD supplemented with 0.5% (w/w) MWEs; 1% MWEs, fed with HFD supplemented with 1% (w/w) MWEs; 2% MWEs, fed with HFD supplemented with 2% (w/w) MWEs. All of the experimental animals were weighted every week. Data were presented as the mean ± SD ( $n = 8$  per group). Values in parentheses are percent body weight change =  $[(BW_t - BW_{t_0}) / BW_{t_0}] \times 100\%$ , where  $BW_{t_0}$  indicates the body weight at week 0 and  $BW_t$  means the body weight at the time  $t$  when euthanized (in units of grams of body weight). <sup>b</sup> Significantly different from HFD ( $p < 0.05$ ). <sup>c</sup> Significantly different from HFD ( $p < 0.01$ ).

**Western Blot Analysis.** Equal amounts of protein samples (50  $\mu$ g) were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and transferred to nitrocellulose membranes (Millipore, Bedford, MA). Membranes were blocked with 5% nonfat milk powder with 0.1% Tween-20 in Tris-buffered saline (TBS) and then incubated with the primary antibody at 4 °C overnight. Afterward, membranes were washed 3 times with 0.1% Tween-20 in TBS and incubated with the secondary antibody conjugated to horseradish peroxidase (GE Healthcare, Little Chalfont, Buckinghamshire, U.K.). Antibodies of FAS and HMG–CoA reductase were from Transduction Laboratory (Lexington, KY) and Upstate Biotechnology (Lake Placid, NY), respectively. Antibodies of PPAR $\alpha$  and CPT-1 were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Band detection was thereafter revealed by enhanced chemiluminescence using electrochemiluminescence (ECL) western blotting detection reagents and exposed in Fujifilm Las-3000 (Tokyo, Japan). Protein quantitative was determined by densitometry using Fujifilm-Multi Gauge, version 2.2, software.

**Statistical Analysis.** In the animal experiments, all data collected were analyzed using an unpaired  $t$  test after one-way analysis of variation (ANOVA) testing, showing a significant difference among all of the groups ( $p < 0.05$ ). Data were analyzed using an unpaired  $t$  test and represented as the mean ± standard deviation (SD). A value of  $p < 0.05$  was considered statistically significant.

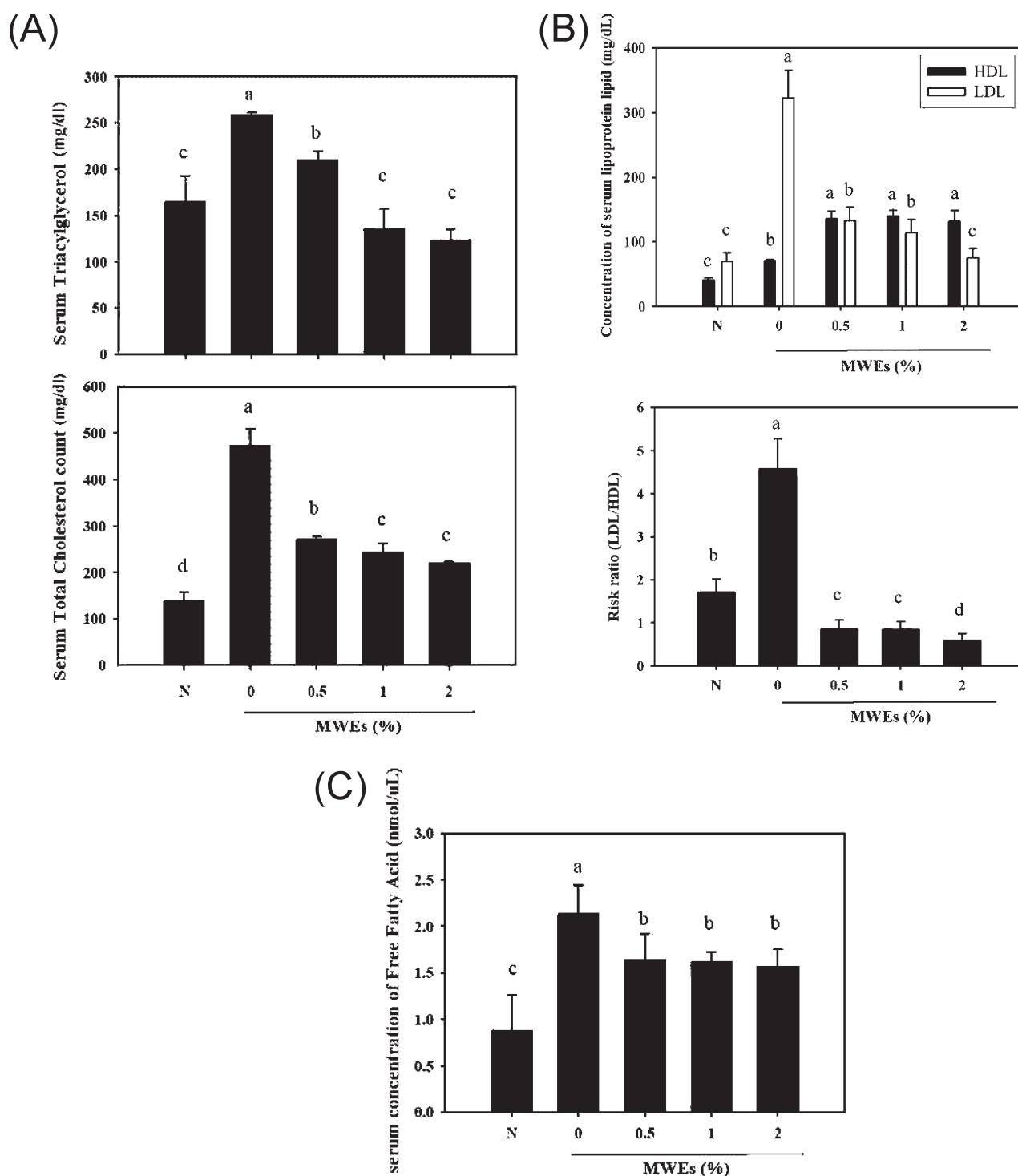
## RESULTS

**Composition of MWEs.** Figure 1 and Table 1 show that the composition of mulberry fruit contains gallic acid, protocatechuic acid, 3-caffeoylquinic acid, chlorogenic acid, 4-caffeoyl quinic acid, caffeic acid, rutin, and quercetin-3- $O$ -glucoside. In addition, our study group has previously reported that mulberry contains about 2.5% anthocyanins, including cyanidine-3-glucoside, cyanidine-3-rutinoside, pelargonidin-3-glucoside, and pelargonidine-3-rutinoside.<sup>15</sup>



**Figure 2.** Effect of MWEs on the ratios of visceral body fat. After the experimental animals were sacrificed by decapitation, the gonadal and perirenal body fat were removed and weighted to obtain the ratio of (A) gonadal body fat/body weight or (B) perirenal body fat/body weight. Data were presented as the mean  $\pm$  SD ( $n = 8$  per group) and analyzed with ANOVA and unpaired  $t$  test ( $p < 0.05$ ).





**Figure 3.** Effect of MWEs on the serum lipid levels. Serum of the experimental animals was collected, and the lipid biomarkers were analyzed. Data were presented as the mean  $\pm$  SD ( $n = 8$  per group) and analyzed with ANOVA and unpaired  $t$  test ( $p < 0.05$ ). (A) Serum levels of TG and cholesterol. (B) Serum levels of LDL-C, HDL-C, and the ratio of LDL/HDL. (C) Serum level of FFA.

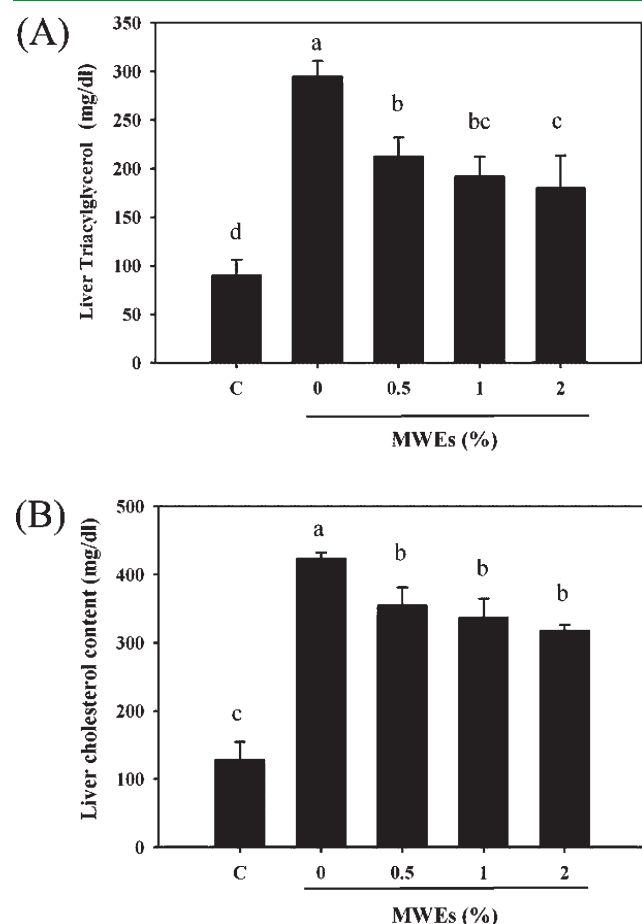
**MWEs Retarded the Body Weight Gain Induced by the HFD.** The body weight gain of the HFD-fed group was 1.4-fold higher as compared to that of the control at the second week and consistently increased until the end of the experiment. The treatment of different doses of MWEs retarded the body weight increase induced by HFD, especially after 10 weeks (Table 2).

These results suggested that MWEs could have potential for body weight reduction.

**MWEs Decreased the Visceral Body Fat.** As shown in Figure 2A, the HFD increased the size and weight ratio of gonadal fat more than 30%. Treatment of MWEs significantly decreased the ratio of gonadal fat, although without a dose-dependent

effect. Similar results were found in Figure 2B. The HFD increased almost 50% of pararenal fat, while MWEs reduced its ratio in a dose-dependent manner. Therefore, MWE was demonstrated to be capable of reducing body fat.

**MWEs Decreased the Serum Lipid Levels.** The serum level of TG increased more than 50% in the HFD-fed group, while



**Figure 4.** Effect of MWEs on the hepatic lipid levels. Livers of the experimental animals were collected, and their lipid contents were then extracted and analyzed. Data were presented as the mean  $\pm$  SD ( $n = 8$  per group) and analyzed with ANOVA and unpaired  $t$  test ( $p < 0.05$ ). (A) Hepatic content of TG. (B) Hepatic content of cholesterol.

MWEs decreased its level dose dependently. As shown in Figure 3A, 1 and 2% of MWEs completely inhibited the TG elevation as compared to the control.

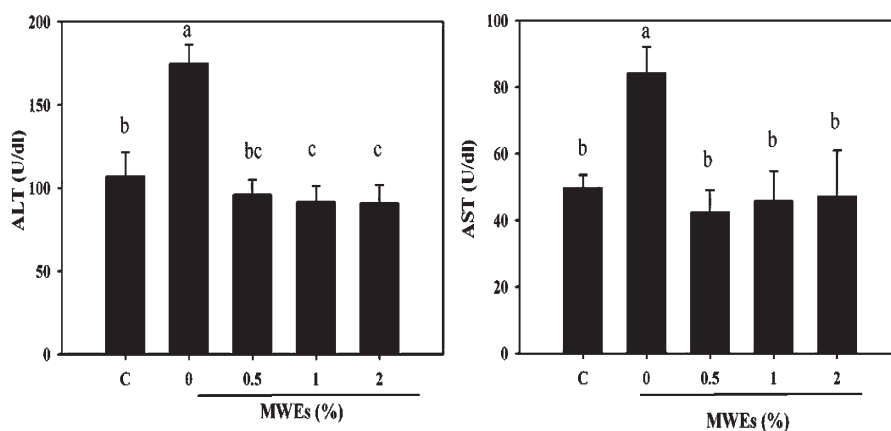
The serum total cholesterol of the HFD-fed group reached more than 3-fold compared to the control. The 0.5% MWEs reduced 40% of serum cholesterol and 60% of LDL-C compared to the HFD group (panels A and B of Figure 3). The 2% MWEs almost ameliorated the effect of HFD and reversed LDL-C to the normal level. Meanwhile, although HFD per se enhanced serum HDL-C slightly, treatment of MWEs could distinguishingly increase HDL-C, which reached 2- and 4-fold compared to that of the HFD and control groups, respectively (Figure 3B). MWEs significantly improved the ratio of LDL/HDL.

The serum FFA level of HFD-fed rats was 2.5-fold compared to the control. Treatment of MWEs significantly reduced 25% of serum FFA compared to the HFD group (Figure 3C). Because the serum FFA levels and other lipid biomarkers are highly associated with the hepatic regulation, the liver lipid content was then analyzed in the following experiment.

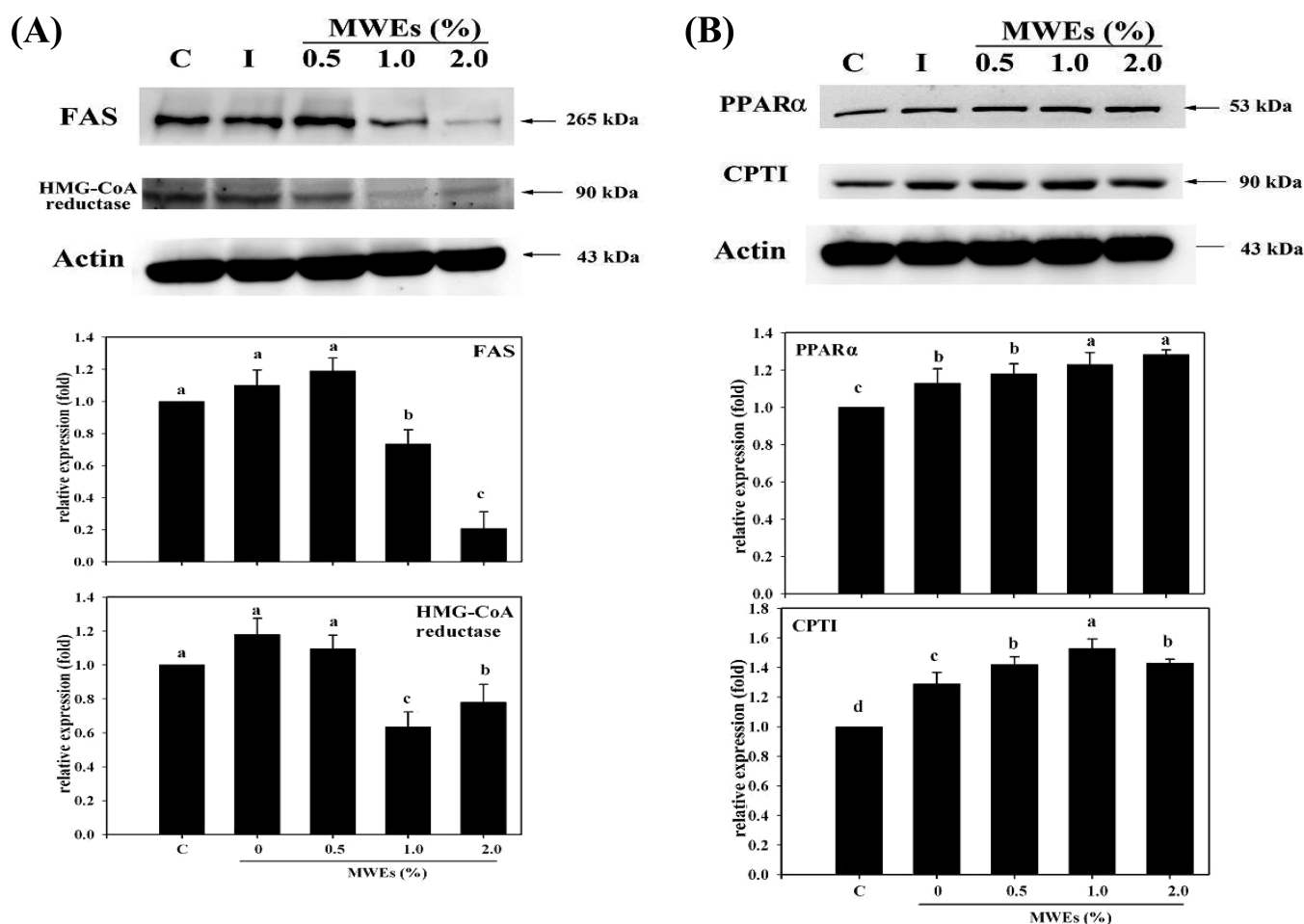
**MWEs Prevented Liver Injury via Reducing the Hepatic Content of TG and Cholesterol.** Figure 4 shows that the hepatic Content of TG and cholesterol of the HFD group was about 3-fold compared to the control, and MWEs dose-dependently reduced TG and cholesterol of the liver. While the HFD increased the levels of ALT and AST, MWEs significantly improved the impairment of hepatic function (Figure 5).

**MWEs Suppressed Hepatic Lipid Synthesis and Enhanced Hepatic Lipid Degradation.** The hepatic expression of FAS and HMG-CoA reductase were significantly decreased by MWEs with concentration above 1% (Figure 6A). In contrast, PPAR $\alpha$  and CPT-1, designated as lipid degradation markers, were elevated by MWE treatment (Figure 6B). These results were compatible with the lipid metabolic changes observed in Figures 4 and 5. As a result, the serum level of FFA and other lipid biomarkers should be highly expected to be associated with the hepatic regulation of lipogenesis and lipolysis.

**No Physiological Burdens Exerted by MWE Administration.** Serum blood urea nitrogen (BUN) and creatinine were not altered by either HFD or MWEs, indicating that neither HFD nor MWEs was renal-toxic to the experimental animals. In addition, the physiological concentrations of sodium and potassium ions were not altered in the whole experiments (data not shown).



**Figure 5.** Effect of MWEs on the expressions of AST and ALT. Serum of the experimental animals was collected, and AST and ALT were analyzed. Data were presented as the mean  $\pm$  SD ( $n = 8$  per group) and analyzed with ANOVA and unpaired  $t$  test ( $p < 0.05$ ).



**Figure 6.** Effect of MWEs on the biomarkers regulating lipid synthesis and degradation. Livers of the experimental animals were collected, and their protein contents were then extracted and analyzed. Data were presented as the mean  $\pm$  SD ( $n = 8$  per group) and analyzed with ANOVA and unpaired  $t$  test ( $p < 0.05$ ). (A) FAS and HMG-CoA reductase were indicated as the markers of lipid synthesis. (B) PPAR $\alpha$  and CPT-1 were indicated as the markers of lipid degradation.

## DISCUSSION

In the present investigation, MWEs showed their good ability in lowering body weight and body fat, accompanied with the hypolipidemic effects, as revealed by reducing serum levels of TG, cholesterol, and the ratio of LDL/HDL. MWEs decreased the hepatic contents of TG and cholesterol and, thus, protected livers from HFD-induced dysfunction. MWEs increased the hepatic expression of PPAR $\alpha$  and CPT-1 and decreased the expression of FAS and HMG-CoA reductase, suggesting that the reduction of serum FFA should be associated with the hepatic regulations of lipid metabolism. Noticeably, all of the effects exerted by MWEs did not cause harm or physiological burden *in vivo*.

The nutrient compositions of MWEs were demonstrated as protein (19.3%), fat (0%), and carbohydrate (54.4%). The total polyphenolic content is about 10.3%.<sup>13</sup> MWE extracted from the whole fruit did show its anti-obesity and anti-dyslipidemia properties, which is supposed to be attributed to the contained polyphenols. The supplementation of synthetic gallic acid and linoleic acid ester might have a potential hypolipidemic effect.<sup>16</sup> The intake of rutin could be beneficial for the suppression of the HFD-induced dyslipidemia, hepatosteatosis, and oxidative stress.<sup>17</sup> Chlorogenic acid exhibited anti-obesity properties and

improved the lipid metabolism in HFD-induced obese mice.<sup>18</sup> In addition to the various polyphenolic compositions, these effects could also be attributed to the water-soluble anthocyanins. In the previous report, MWEs and mulberry anthocyanin-rich extracts (MACs) possessed antioxidative and anti-atherosclerosis abilities *in vitro*.<sup>15</sup> Both MWEs and MACs showed a great ability of scavenging free radicals, inhibiting LDL oxidation, and decreasing atherogenic stimuli in macrophages, while the efficacy of MACs was 10-fold greater than that of MWEs. These results demonstrated that anthocyanic components in mulberry extracts were regarded as indispensable in the prevention of atherosclerosis. Mulberry has been identified to be rich in anthocyanins, including cyanidine-3-glucoside, cyanidine-3-rutinoside, pelargonidine-3-glucoside, and pelargonidine-3-rutinoside. In the previous report, Prior et al. demonstrated that feeding purified anthocyanins from blueberries or strawberries reduced the body weight and body fat of rats fed with a HFD.<sup>19</sup>

The effects of MWEs on hepatic enzyme expressions suggested that MWEs could exert a hypolipidemic effect via inhibiting lipid synthesis and promoting lipid degradation. The mitochondrial  $\beta$ -oxidation of long-chain fatty acids is initiated by the sequential action of CPT-1 (outer membrane and detergent labile) and CPT-2 (inner membrane and detergent



stable), together with the carnitine carrier.<sup>20</sup> CPT-1 catalyzes the first reaction in the transport of long-chain fatty acids from the cytoplasm to the mitochondrion, a rate-limiting step in  $\beta$ -oxidation. Hence, CPT-1 was frequently considered as one of the markers of lipid degradation.

PPAR is one of the family of ligand-activated transcription factors, which are classified as  $\alpha$ ,  $\beta/\delta$ , and  $\gamma$ . Among the three isoforms, PPAR $\alpha$  is expressed abundantly in liver, kidney, and skeletal muscle.<sup>21</sup> The activation of PPAR $\alpha$  mediates lipoprotein metabolism, enhances hepatic uptake, and increases the esterification of FFAs. Moreover, it promotes mitochondrial fatty acid uptake and oxidation.<sup>22</sup> The PPAR $\alpha$ -regulated pathway is enhanced in a diabetic heart, which relies primarily on fatty acid oxidation for energy demand.<sup>23</sup> In the present study, the expression of PPAR $\alpha$  was dose-dependently increased by MWEs. Although PPAR $\alpha$  could mediate the fatty acid oxidation synergistically with CPT-1, the other metabolic aspects regulated by PPAR $\alpha$  should still not be excluded.

The increase of serum FFA is considered to be associated with obesity and its related metabolic complications, such as dyslipidemia and insulin resistance. Our data showed that MWEs lowered the serum FFA, along with body fat reduction and the lipidemic alteration. It was reported that the total FFA rate of appearance ( $R_a$ ) increased linearly with increasing fat mass, with no differences between men and women.<sup>24</sup> Koutsari and Jensen indicated that, although women have greater FFA release rates than men, a higher FFA level or a greater fatty acid oxidation were not found in female.<sup>25</sup> These findings implicated that women have greater non-oxidative FFA disposal to avoid the potential FFA harm. It was demonstrated that greater FFA was taken up in women than in men.<sup>26</sup> The high FFA levels were shown to modulate microvascular function and contribute to obesity-associated hypertension and microangiopathy.<sup>27</sup> FFA-associated insulin resistance could be mediated by the molecular mechanism transduced by FFA in  $\beta$  cells involving the expression of GPR40 and IRS-1 serine kinase. The former played a critical role in the development of insulin resistance, while the latter regulated the negative feedback loop of the insulin receptor signal pathway.<sup>28</sup>

In view of the distribution of body fat, there existed the controversies of whether visceral or subcutaneous fat contributed to the high-serum FFA? It was suggested that total body fat but not regional fat distribution nor sex difference is the most important modulator of FFA release.<sup>24</sup> Another previous study showed that upper body fat is more lipolytic than lower body fat in both men and women. Because visceral fat only accounts for a small amount of total body fat, upper body subcutaneous fat was suggested as the dominant contributor to the excess of circulating FFA.<sup>25</sup> However, other investigations revealed different results. Kim et al. measured the fasting levels of serum FFA, insulin, glucose disappearance rate, and hepatic glucose production after surgical removal of visceral or subcutaneous fat tissue in monosodium glutamate-obese rats. They demonstrated that visceral fat affected the FFA level and insulin sensitivity more than subcutaneous fat.<sup>29</sup> Furthermore, the redistribution of body fat by reducing visceral fat and increasing the subcutaneous fat could be protective from metabolic syndrome.<sup>30</sup> In the present study, treatment of MWEs reduced the pararenal and gonadal fat consistently with the body weight reduction; nevertheless, the redistribution of body fat could still not be ruled out. These results implicated that MWEs possess the potential of improving the obesity-related metabolic syndromes.

In conclusion, MWE is suggested to be a harmless and effective anti-obese agent, which can be used to reduce body weight, serum, and liver lipids.

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### Author Contributions

<sup>†</sup>These authors contributed equally to this work and, therefore, share the first authorship.

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