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FULL COMMUNICATIONS

Combined Treatment of Mulberry Leaf and Fruit Extract Ameliorates Obesity-Related Inflammation and Oxidative Stress in High Fat Diet-Induced Obese Mice

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ABSTRACT The aim of this study was to investigate whether a combined treatment of mulberry leaf extract (MLE) and mulberry fruit extract (MFE) was effective for improving obesity and obesity-related inflammation and oxidative stress in high fat (HF) diet-induced obese mice. After obesity was induced by HF diet for 9 weeks, the mice were divided into eight groups: (1) lean control, (2) HF diet-induced obese control, (3) 1:1 ratio of MLE and MFE at doses of 200 (L1:1), (4) 500 (M1:1), and (5) 1000 (H1:1) mg/kg per day, and (6) 2:1 ratio of MLE and MFE at doses of 200 (L2:1), (7) 500 (M2:1), and (8) 1000 (H2:1) mg/kg per day. All six combined treatments significantly lowered body weight gain, plasma triglycerides, and lipid peroxidation levels after the 12-week treatment period. Additionally, all combined treatments suppressed hepatic fat accumulation and reduced epididymal adipocyte size. These improvements were accompanied by decreases in protein levels of proinflammatory markers (tumor necrosis factor-alpha, C-reactive protein, interleukin-1, inducible nitric oxide synthase, and phospho-nuclear factor-kappa B inhibitor alpha) and oxidative stress markers (heme oxygenase-1 and manganese superoxide dismutase). M2:1 was the most effective ratio and dose for the improvements in obesity, inflammation, and oxidative stress. These results demonstrate that a combined MLE and MFE treatment ameliorated obesity and obesity-related metabolic stressors and suggest that it can be used as a means to prevent and/or treat obesity.

KEY WORDS: • *inflammation* • *mulberry fruit* • *mulberry leaf* • *obesity* • *oxidative stress*

INTRODUCTION

OBESITY, WHICH IS EXCESSIVE FAT accumulation in adipose tissue and dramatic changes in fat distribution in the body, has increased globally in recent decades. Obesity causes various health problems, such as diabetes, cardiovascular diseases, dyslipidemia, and cancers.[1] Adipose tissue produces various hormones and adipokines and is an important organ involved in the metabolic complications of obesity. Free fatty acids (FFAs) released from adipose tissue contribute to insulin resistance by impairing the insulin-signaling pathway as well as lipid metabolism.[2] One of the most responsive tissues to excessive FFAs is liver. When hepatic influx of FFAs is increased, it becomes toxic to hepatic cells and results in membrane disruption, mitochondrial dysfunction, and alterations in intracellular signaling pathways.[3–5] From a pathophysiology perspective, it causes hepatic insulin resistance and hepatic steatosis, which can progresses to severe stages of non-alcoholic fatty liver disease.[6] Excess FFAs can also increase the oxidative stress response, which is a process related to obesity, cardiovascular alterations, and cancers.[7] Moreover, the expansion of fat mass leads to macrophage infiltration in adipose tissue and the production of proinflammatory cytokines accompanied by decreases in anti-inflammatory cytokines, which is ultimately associated with the development of obesity-related comorbidities.[8,9] The dysregulation of adipokines and infiltration of inflammatory cells in adipose tissue contributes to chronic low-grade inflammation and oxidative stress.[10] Eventually, oxidative stress and systemic inflammation cause endothelial cell dysfunction, resulting in insulin resistance, diabetes, and atherosclerosis.[11]

Most current antiobesity drugs have potential side effects. Thus, many studies have focused on natural compounds that have no adverse side effects but have potential benefits for obese patients. Mulberry leaf and fruit have been a part of traditional oriental medicine for a long time and have been suggested to be beneficial for preventing or treating obesity, diabetes, and dyslipidemia.[12–16] A previous study reported that the ethanol extract of mulberry leaves decreases body weight and acts as a melanin-concentrating hormone-1 antagonist in diet-induced obese mice.[12] Several studies have also demonstrated that mulberry leaves inhibit activation of

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nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) induced by tumor necrosis factor alpha (TNF-α) in vascular endothelial cells[17] and control the inflammatory response and oxidative stress by suppressing the production of nitrite and thiobarbituric acid (TBA) reactive substances in blood and tissues.[18] Anthocyanins in mulberry fruit can scavenge free radicals and inhibit low-density lipoprotein (LDL) oxidation.[19] Additionally, mulberry fruit ameliorates inflammation in lipopolysaccharide (LPS)-stimulated mouse primary macrophages and arthritic rats.[20,21]

To date, most of the existing therapeutic evidence is based on experiments of a single dose or type of mulberry leaf or fruit, and the beneficial effects of a mulberry leaf and fruit cocktail have not been explored previously in obesity research. We hypothesized that a mulberry leaf and fruit cocktail may have beneficial effects on preventing or treating obesity by modulating the inflammatory response and oxidative stress. To test this hypothesis, the responses of obesity, inflammation, and oxidative stress to various ratios and doses of mulberry leaf extract (MLE) and mulberry fruit extract (MFE) were compared in a rodent model of high fat (HF) diet-induced obesity.

MATERIALS AND METHODS

Plant material and extraction

Mulberry leaves and fruits were collected from Yang-Pyeong Agricultural Development and Technology Center (YangPyeong-gun, South Korea). The dried leaves and fruits were extracted with 70% ethanol. The mixture was filtered, evaporated in a rotary evaporator, and lyophilized. The yield was 20% and 28% of the starting dry weight of mulberry leaves and fruits, respectively.

Animals

Male C57BL/6 mice (Orient Bio, Seongnam, South Korea) were housed at a constant temperature (22 ± 1°C) with a 12 h dark/light cycle with access to water and food *ad libitum*. After a one week acclimation period, the mice were randomly divided into two groups: (1) control diet and (2) HF diet. Each group was fed with either control diet (D12450B, 10% kcal fat; Research Diets, New Brunswick, NJ, USA) or HF diet (D12451, 45% kcal fat; Research Diets) for 9 weeks, respectively.

Experimental design

After obesity was induced by the HF diet for 9 weeks, the mice were divided into eight groups ($n = 6$ per group) as follows: (1) lean control (CON), (2) HF diet-induced obese control (HF), (3) HF diet at a 1:1 ratio of MLE and MFE at a dose of 200 (L1:1), (4) 500 (M1:1), and (5) 1000 (H1:1) mg/kg per day, and (6) HF diet at a 2:1 ratio of MLE and MFE at a dose of 200 (L2:1), (7) 500 (M2:1), and (8) 1000 (H2:1) mg/kg per day. Treatments were administered daily via stomach gavage for 12 weeks. Body weights and food intake were recorded during the treatment period. At the end of the experimental period, the animals were anesthetized with intraperitoneal Zoletil/Rompun after an overnight fast and blood was collected from a post-caval vein. Then, tissues were isolated and stored at -80°C until analysis. This study protocol conformed with the National Institutes of Health Guiding Principles for the Care and Use of Laboratory Animals and was approved by the Institutional Animal Care and Use Committee of Kyung Hee University.

Intraperitoneal glucose tolerance test

After the treatment period, the mice were subjected to an overnight 16-h fast. Then, glucose solution (2 g/kg) was injected intraperitoneally. Blood glucose levels were measured at 0, 15, 30, 60, 90, and 120 min after the glucose challenge using a glucometer.

Biochemical analysis

Plasma triglycerides (TGs), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C) levels were measured enzymatically using commercial kits (Bio Clinical System, Anyang, South Korea). The atherogenic index (AI) was estimated by: AI = (plasma TC – plasma HDL-C)/plasma HDL-C.

Measurement of lipid peroxidation

Levels of malondialdehyde (MDA) were measured as a marker of lipid peroxidation using the TBA method. Liver homogenates (200 μL) with 0.15 M potassium chloride buffer, 0.2 mL of 8.1% sodium dodecyl sulfate, 3 mL of 20% acetic acid–0.8% TBA mixture, and 600 μL of water were added, and then heated at 95°C for 60 min. After cooling on ice, 1 mL of water and 5 mL mixture of n-butanol and pyridine (15:1, v/v) were added, and centrifuged at 2500 *g* for 10 min. The absorbance of the supernatants was measured at 532 nm.

Histological analysis

Histological sections were prepared, and stained with hematoxylin and eosin. Stained sections were observed using an optical microscope (HS-100). The mean size of epididymal adipocytes was measured using Image J software (National Institutes of Health, Bethesda, MD, USA).

Western blot analysis

Liver lysates were prepared, and proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred onto polyvinylidene fluoride membranes. The membranes were blocked, and incubated with antibodies against TNF-α, interleukin-1β (IL-1β), phosphonuclear factor-kappa B inhibitor alpha (p-IKBα), copper-zinc superoxide dismutase (Cu-ZnSOD), glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Santa Cruz Biotechnology, Santa Cruz, CA, USA), C-reactive protein (CRP), glutathione peroxidase (Gpx) (Abcam, Cambridge, United Kingdom), inducible nitric oxide synthase (iNOS), heme oxygenase-1 (HO-1), and manganese SOD (MnSOD; Stressgen, Victoria, BC, Canada). Membranes were then exposed to horseradish peroxidase-conjugated secondary

antibody (Santa Cruz Biotechnology). Detection was performed using enhanced chemiluminescence reagent (Santa Cruz Biotechnology). Western blot images were obtained with luminescent image analyzer (Gbox; Syngene, Frederick, MD, USA), and band intensities were quantified. Densitometry analysis evaluates the relative amount of protein band and quantifies the results in terms of optical density. Levels of protein expression were normalized to values for GAPDH.

Statistical analysis

Data are expressed as mean ± standard error of the mean. Statistical significance was determined by one-way analysis of variance followed by Duncan's multiple comparisons test using SPSS Statistics 20. $P < .05$ was considered statistically significant.

RESULTS

Effects of combined treatment of MLE and MFE on body weight gain, food intake, and food efficiency ratio

The body weight gains in HF diet-fed obese controls increased significantly compared to those of lean controls. Mice treated with MLE and MFE had a lower body weight gain compared with that of HF obese controls, although food intake did not differ among groups. Therefore, the combined MLE and MFE treatments significantly lowered food efficiency ratio (FER) compared with that of HF group, except in the H2:1 group (Table 1).

Effects of combined treatment of MLE and MFE on glucose tolerance

Intraperitoneal glucose tolerance test (IPGTT) was conducted to evaluate insulin sensitivity, and IPGTT results showed impaired glucose tolerance in HF group compared with that in lean controls (Fig. 1A). Further, the area under the curve (AUC) of IPGTT was significantly greater in HF group compared with that in lean controls. However, L1:1, L2:1, and M2:1 treatments significantly reduced AUC of IPGTT compared with that in HF group (Fig. 1B).

Table 1. Effects of Combined Treatment of Mulberry Leaves and Fruit Extracts on Body Weight Gain, Food Intake, and Its Efficiency Ratio in C57BL/6 Mice Fed a High Fat Diet

	Body weight gain (g for 12 weeks)	*Food intake (g/day)*	*FER (%)*
CON	4.42 ± 0.25^{a}	2.44 ± 0.07	2.16 ± 0.12^{a}
HF	12.80 ± 0.85^{d}	2.41 ± 0.12	5.95 ± 0.68^{d}
L1:1	6.93 ± 0.82^{abc}	2.27 ± 0.01	3.64 ± 0.43^{abc}
M1:1	7.73 ± 1.01^{bc}	2.22 ± 0.09	4.15 ± 0.56^{bc}
H1:1	8.59 ± 0.82^{c}	2.41 ± 0.06	4.23 ± 0.39^{bc}
L2:1	5.92 ± 1.29^{abc}	2.22 ± 0.01	3.17 ± 0.68^{abc}
M2:1	5.60 ± 0.92^{ab}	2.50 ± 0.35	2.79 ± 0.53^{ab}
H2:1	8.40 ± 0.80^{bc}	2.16 ± 0.01	4.62 ± 0.43^{cd}

Values are mean ± SEM ($n = 6$).

[abcd]Different letters within a variable are significantly different at $P < .05$.

FER, food efficiency ratio; CON, control diet; L1:1, HF diet with 200 mg/kg per day (1:1) of MLE and MFE; M1:1, HF diet with 500 mg/kg per day (1:1) of MLE and MFE; H1:1, HF diet with 1000 mg/kg per day (1:1) of MLE and MFE; L2:1, HF diet with 200 mg/kg per day (2:1) of MLE and MFE; M2:1, HF diet with 500 mg/kg per day (2:1) of MLE and MFE; H2:1, HF diet with 1000 mg/kg per day (2:1) of MLE and MFE; HF, high fat; MLE, mulberry leaf extract; MFE, mulberry fruit extract; SEM, standard error of the mean.

Effects of combined treatment of MLE and MFE on plasma lipid profiles

Plasma TC levels increased in HF group compared to those in lean controls, but no significant difference was observed between HF group and the treatment groups. No differences in HDL-C levels were found among groups. Plasma TG levels decreased significantly in all treatment groups compared with that in HF group. Moreover, MLE and MFE treatments significantly decreased AI, except in L1:1 group (Table 2).

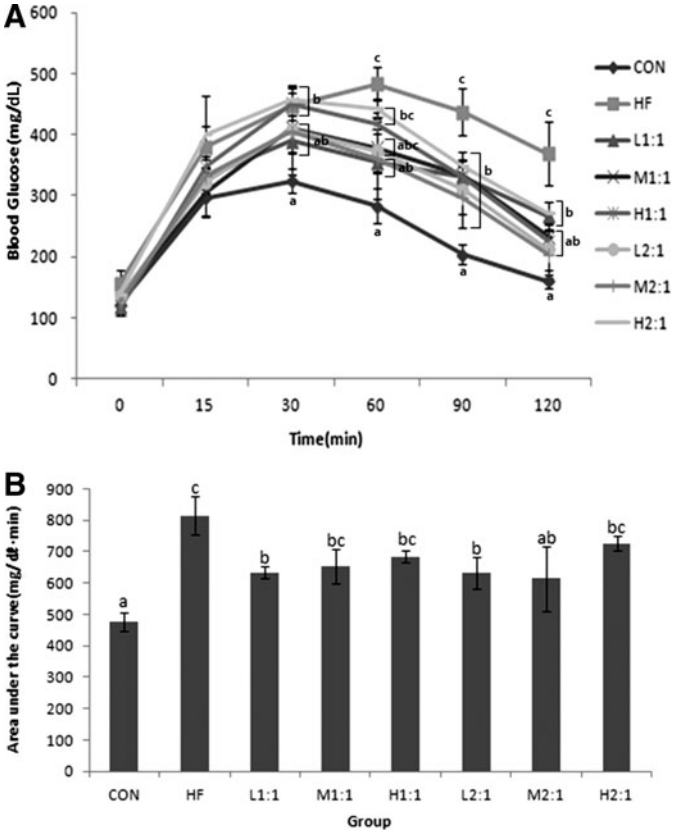

FIG. 1. Effects of combined treatment of mulberry leaves and fruit extracts on **(A)** glucose tolerance and **(B)** the area under the curve of intraperitoneal glucose tolerance test. Values are mean ± SEM ($n = 6$). [abc]Different letters within a variable are significantly different at $P < .05$. CON, control diet; L1:1, HF diet with 200 mg/kg per day (1:1) of MLE and MFE; M1:1, HF diet with 500 mg/kg per day (1:1) of MLE and MFE; H1:1, HF diet with 1000 mg/kg per day (1:1) of MLE and MFE; L2:1, HF diet with 200 mg/kg per day (2:1) of MLE and MFE; M2:1, HF diet with 500 mg/kg per day (2:1) of MLE and MFE; H2:1, HF diet with 1000 mg/kg per day (2:1) of MLE and MFE; HF, high fat; MLE, mulberry leaf extract; MFE, mulberry fruit extract; SEM, standard error of the mean.

Table 2. Plasma Lipid Profiles in C57BL/6 Mice Treated with Combined Treatments of Mulberry Leaves and Fruit Extracts

	TC (mg/dL)	*HDL-C (mg/dL)*	*TG (mg/dL)*	*AI*
CON	106.02 ± 9.06[a]	65.92 ± 5.62	69.98 ± 5.46[a]	0.66 ± 0.03[a]
HF	163.38 ± 8.77[b]	62.03 ± 6.38	154.37 ± 14.51[b]	1.54 ± 0.32[b]
L1:1	137.90 ± 6.60[ab]	69.11 ± 6.58	87.14 ± 19.39[a]	1.09 ± 0.15[ab]
M1:1	130.54 ± 12.98[ab]	74.76 ± 5.50	82.90 ± 13.25[a]	0.79 ± 0.21[a]
H1:1	137.46 ± 9.24[ab]	67.29 ± 5.07	65.43 ± 7.45[a]	0.94 ± 0.22[a]
L2:1	143.99 ± 13.81[b]	73.86 ± 5.62	90.20 ± 9.47[a]	0.95 ± 0.12[a]
M2:1	137.28 ± 13.40[ab]	77.04 ± 8.85	93.37 ± 11.62[a]	0.74 ± 0.10[a]
H2:1	140.19 ± 4.74[ab]	75.17 ± 1.91	93.32 ± 12.69[a]	0.91 ± 0.10[a]

Values are mean ± SEM ($n = 6$).

[ab]Different letters within a variable are significantly different at $P < .05$.

TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; AI, atherogenic index.

Effects of combined treatment of MLE and MFE on liver lipid peroxidation

Levels of MDA, as an indicator of lipid peroxidation, were significantly higher in HF group compared with those in lean controls. However, MDA levels decreased to the level of the lean controls in all treatment groups (Table 3).

Effects of combined treatment of MLE and MFE on liver and adipose tissue morphology

Histological analyses showed that livers in HF group had macrovesicular and microvesicular steatosis, whereas the MLE and MFE treatments less accumulation of liver fat (Fig. 2A). Moreover, hepatic fat accumulation was significantly suppressed by treatment with a 2:1 ratio of MLE and MFE more than by treatment with a 1:1 ratio of MLE and MFE (Fig. 2A). The histological examination of epididymal adipose tissue showed that adipocyte size was large and numerous immature adipocytes were observed in HF group (Fig. 2B). In contrast, MLE and MFE treatments considerably suppressed adipocyte size and the number of immature adipocytes (Fig. 2C).

Table 3. Effects of Combined Treatment of Mulberry Leaves and Fruit Extracts on Lipid Peroxidation in Livers of C57BL/6 Mice Fed a High Fat Diet

	MDA concentration (nM)
CON	18.51 ± 0.79[a]
HF	25.31 ± 3.38[b]
L1:1	17.22 ± 1.11[a]
M1:1	17.98 ± 1.93[a]
H1:1	18.00 ± 1.93[a]
L2:1	17.33 ± 2.66[a]
M2:1	18.04 ± 1.75[a]
H2:1	17.46 ± 1.71[a]

Values are mean ± SEM ($n = 6$).

[ab]Different letters indicate significant difference at $P < .05$.

MDA, malondialdehyde.

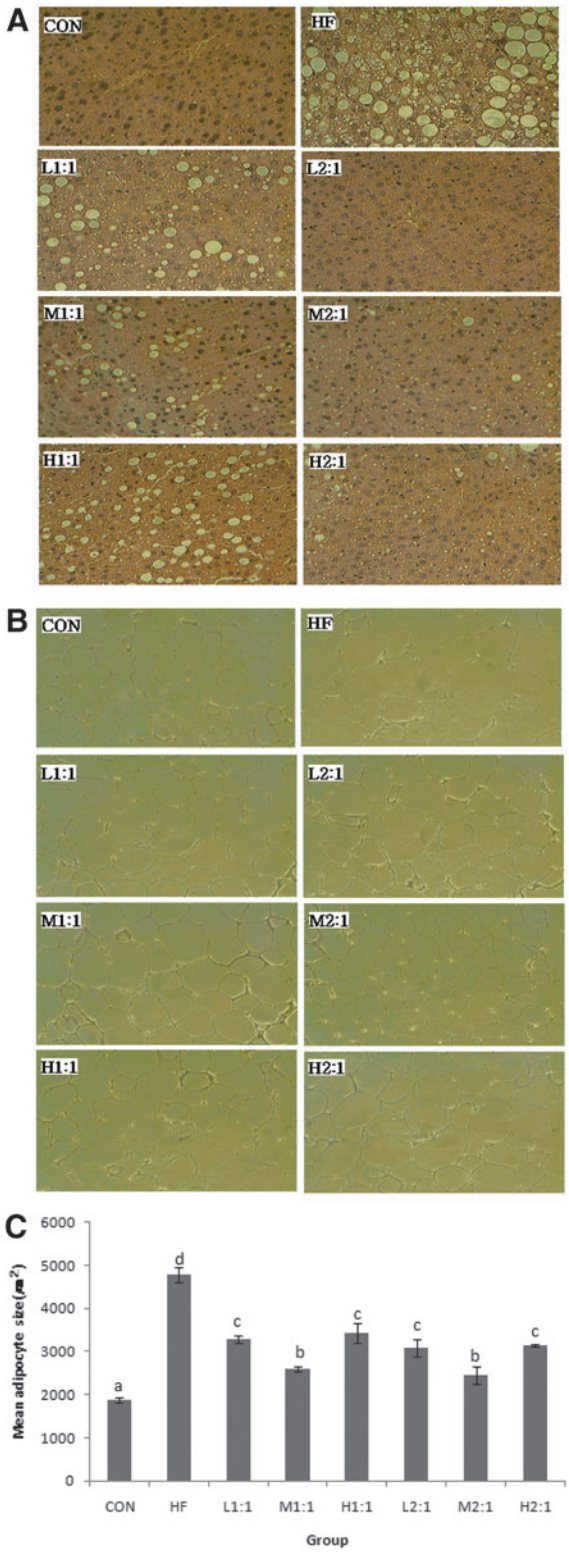

FIG. 2. Histological analysis of **(A)** liver (original magnification, 40×) and **(B)** epididymal adipose tissues (original magnification, 20×), and **(C)** quantification of epididymal adipocyte size. Values are mean ± SEM ($n = 6$). [abcd]Different letters within a variable are significantly different at $P < .05$. Color images available online at www.liebertpub.com/jmf

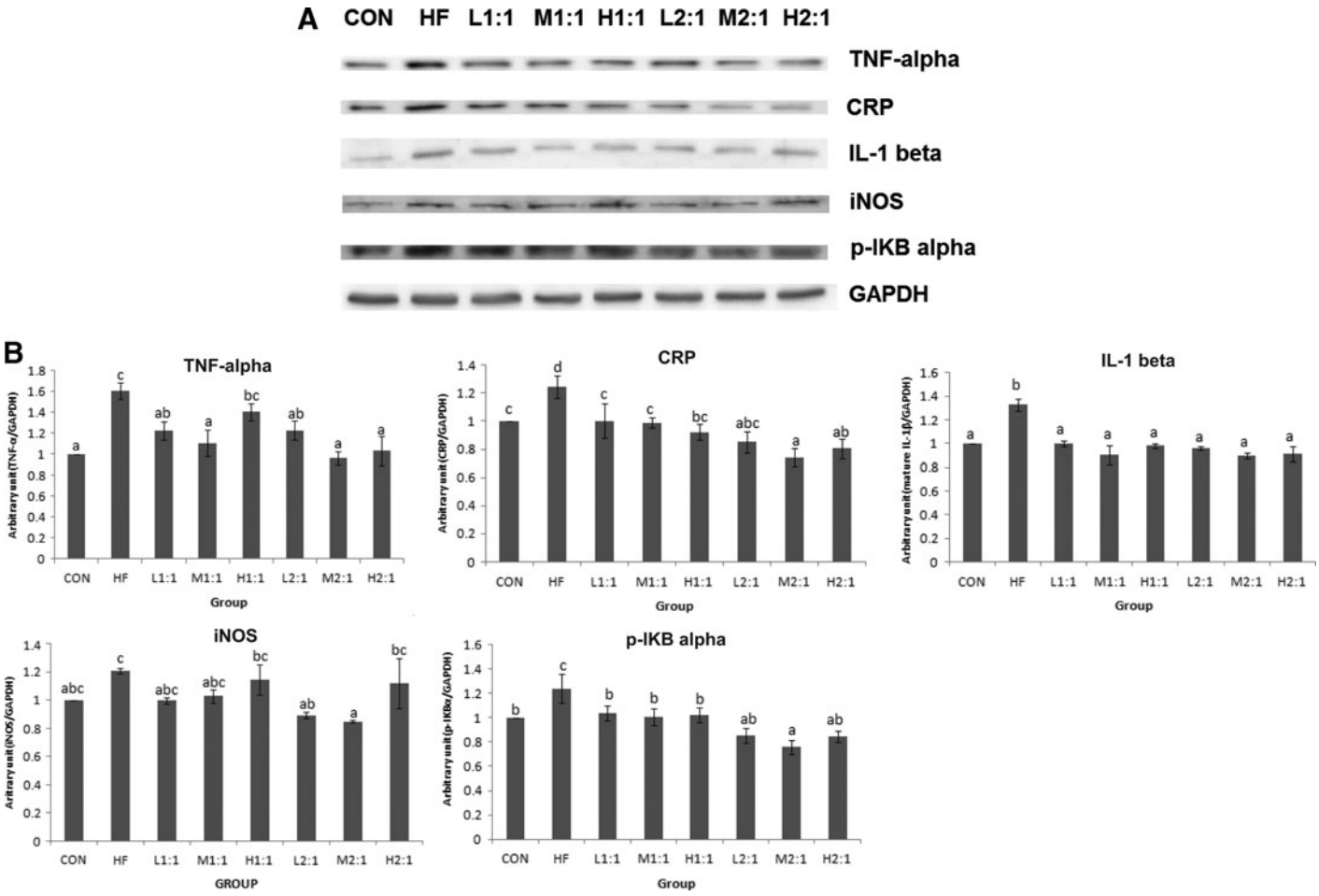

FIG. 3. Effects of combined treatments of mulberry leaves and fruit extracts on proinflammatory markers in livers: **(A)** representative Western blots for proinflammatory markers. **(B)** Densitometric analysis of tumor necrosis factor alpha (TNF-α), C-reactive protein (CRP), interleukin-1β (IL-1β), inducible nitric oxide synthase (iNOS), and phospho-nuclear factor-kappa B inhibitor alpha (p-IKBα). Levels of protein expression were normalized to values for glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Values are mean ± SEM ($n = 6$). [abcd]Different letters within a variable are significantly different at $P < .05$.

Effects of combined treatment of MLE and MFE on liver inflammatory markers

To investigate whether a combined MLE and MFE treatment altered inflammation in liver, proinflammatory markers were analyzed. Protein levels of liver TNF-α increased in HF group compared with those in lean controls. However, MLE and MFE treatments significantly decreased levels of liver TNF-α, except in H1:1 group (Fig. 3). Liver CRP levels were higher in HF group than those of lean controls, but the levels were markedly decreased following MLE and MFE treatments. Liver CRP levels in M2:1 group were the lowest among all treatment groups. The mature IL-1β levels in liver were higher in HF group compared with those in lean controls. In contrast, liver IL-1β levels decreased to the control levels in all treatment groups. Although liver iNOS levels were not significantly different between lean controls and HF group, it decreased significantly in L2:1 and M2:1 groups. Liver p-IKBα levels increased in HF group compared with those in lean controls, but these levels decreased considerably in all treatment groups. The decreases in p-IKBα levels were greater in 2:1 ratio groups than those in 1:1 ratio groups. The p-IKBα levels in M2:1 group was the lowest among all treatment groups.

Effects of combined treatment of MLE and MFE on oxidative stress marker protein levels

The effects on oxidative stress markers were analyzed in livers of mice treated with various ratios and doses of MLE and MFE. As shown in Figure 4, protein levels of HO-1 were significantly greater in HF group than those in lean controls. M2:1 treatment significantly decreased HO-1 levels compared with those in HF group. Although MnSOD levels were not different between lean controls and HF group, levels decreased in M2:1 treatment compared with those in HF group. No significant differences in the levels of Cu-ZnSOD (Fig. 4) or Gpx were observed among all groups (data not shown).

DISCUSSION

This study investigated the effects of a combined treatment of MLE and MFE on obesity and obesity-associated

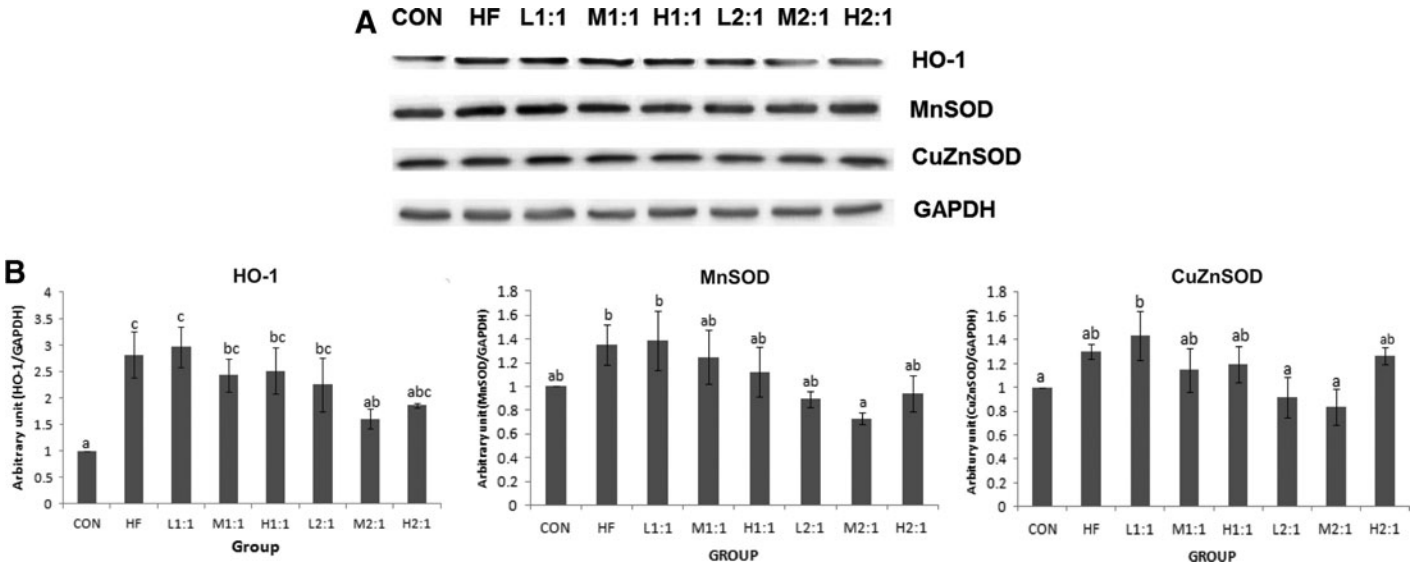

FIG. 4. Oxidative stress markers in livers of C57BL/6 mice with combined treatments of mulberry leaves and fruit extracts: **(A)** representative Western blots for oxidative stress markers. **(B)** Densitometric analysis of heme oxygenase-1 (HO-1), manganese superoxide dismutase (MnSOD), and copper-zinc SOD (CuZnSOD). Levels of protein expression were normalized to values for GAPDH. Values are mean ± SEM ($n = 6$). [abc]Different letters within a variable are significantly different at $P < .05$.

metabolic stressors. The combined treatments were effective for improving obesity-related phenotypes as shown by the lower body weight gain and epididymal adipocyte size. Additionally, indices of inflammation and oxidative stress were altered toward ameliorating those stressors. These findings demonstrate that the combined MLE and MFE treatments were beneficial for treating obesity and its related phenotypes in mice.

The combined MLE and MFE treatments significantly decreased body weight gain and FER compared with HF group, suggesting that the MLE and MFE treatments were responsible for body weight loss despite no difference in food intake. IPGTT results showed improvements in insulin resistance with 1:1 and 2:1 ratios of MLE and MFE. Previous studies have demonstrated that mulberry leaves, which include 1-deoxynojirimycin, have an α-glucosidase inhibitory effect[22] and play an important role in the management of diabetes by regulating hepatic gluconeogenesis,[23] suggesting an antidiabetic effect for mulberry leaves. Flavonoids in mulberry leaves reduce plasma TG, TC, and LDL cholesterol in high cholesterol diet-induced hyperlipidemic rats.[24,25] Moreover, mulberry fruit improves blood lipid profiles and lipid metabolism in hyperlipidemic rats.[26] Our results similarly showed that the combined MLE and MFE treatments significantly reduced plasma TG levels and decreased atherogenicity as shown by a reduced AI compared with those in HF group, except in L1:1. These results indicate that the combined MLE and MFE treatments might have additive or synergistic benefits for improving TG and cholesterol metabolism in obese rats.

Increased liver TG depots cause further fat accumulation in hyperlipidemic mice.[27] Our histological examination showed that livers in HF group developed macrovesicular and microvesicular steatosis, whereas rats fed the 1:1 and 2:1 ratios of MLE and MFE revealed decreased liver fat accumulation. Further, adipocyte hypertrophy was inhibited by the MLE and MFE treatments, which was consistent with previous findings that the three herbs (*Melissa officinalis L.*, *Morus alba L.*, and *Artemisia capillaris Thunb*) decrease adipose tissue mass by inhibiting angiogenesis in nutritionally induced obese mice.[28] These results suggest that the MLE and MFE treatments effectively suppressed fatty liver and adipocyte hypertrophy.

Adipocyte hypertrophy stimulates macrophage infiltration and enhances the inflammatory response through the production of reactive oxygen species (ROS) and inflammatory cytokines.[8] Excessive FFAs and saturated fatty acids from adipose tissue lead to fat accumulation in the liver and other tissues, resulting in an increased inflammatory reaction.[10] Fat accumulation in the liver increases LDL overproduction together with inflammatory cytokines, such as IL-6 and CRP.[29] Proinflammatory cytokines, including TNF-α, IL-6, iNOS, and CRP are elevated in obese subjects.[30] Our results show that liver levels of TNF-α, iNOS, and CRP increased in HF group but were selectively decreased by the combined MLE and MFE treatments. We also showed that liver iNOS levels decreased by treatment with L2:1 and M2:1. A previous study supported that MLE suppresses inflammatory mediators, including NO, prostaglandin E2, and cytokines by inhibiting iNOS activity in macrophages.[31] IL-1β plays a role as a crucial inflammation mediator in obesity. Our result showed that liver IL-1β levels were significantly increased in HF group; however, MLE and MFE treatments lead to decrease liver IL-1β levels. Previous studies have demonstrated that TNF-α and LPS stimulate NF-κB activation, which is a key transcription factor of proinflammatory genes.[32] Translocation of NF-κB into the nucleus is preceded by IκBα phosphorylation.[33] We observed increased p-IκBα as an indirect marker of NF-κB activation

in HF group, whereas these levels decreased following the combined MLE and MFE treatments.

Adipose tissue, as a motor for inflammation related to obesity, leads to the production of ROS and adipokines as well as the activation of inflammatory genes, resulting in inhibited insulin signaling.[29] Increased oxidative stress, as demonstrated by elevated MDA levels, was observed in the HF group compared to lean controls. In contrast, MDA levels decreased to control levels in all treatment groups. Therefore, MLE and MFE suppressed ROS production mediated by oxidative stress associated with obesity. Western blot analyses of oxidative stress markers demonstrated that HO-1, which is activated by oxidative stress,[34] increased in the HF group compared with that in lean controls, whereas HO-1 levels decreased significantly following treatment with M2:1. MnSOD levels were not significantly different between lean controls and HF group, whereas MnSOD levels decreased in M2:1 group. Among the tested oxidative stress indices, levels of Cu-ZnSOD and Gpx were not altered by the treatments, suggesting that HO-1 and MnSOD were more responsive to the MLE and MFE treatment than Cu-ZnSOD and Gpx.[35,36] Further, these data suggest that the expression of antioxidant enzymes increased to remove excess ROS production in HF group, whereas antioxidant supplements, such as MLE and MFE lead to downregulation of antioxidant enzymes due to reduced oxidative stress. To date, many studies have shown no consistent pattern in endogenous antioxidant expression.[37–39] Therefore, further experiments, including other key antioxidant system mediators, such as nuclear factor erythroid 2-related factor and the activities of antioxidant enzymes in various tissues are required to confirm the antioxidative effects of mulberry.

Our previous study demonstrated that a combined MLE and MFE treatment possesses higher antiobesity effects than those of a single treatment with MLE. Thus, in the present study, different ratios and doses of MLE and MFE in combination were applied to HF diet-induced obese mice to determine the best ratio and dose for treating obesity and decreasing its related stressors. In conclusion, the combined MLE and MFE treatments were effective against obesity and its related inflammation and oxidative stress. The 2:1 ratio of MLE and MFE was better than the 1:1 ratio for the biochemical improvements. The medium dose (500 mg/kg per day) among the 2:1 ratio groups was the best to treat obesity and its related inflammation and oxidative stress. Additionally, the high dose (1000 mg/kg per day) cannot be recommended due to a selective adverse effect on obesity. Taken together, we suggest that a combined MLE and MFE treatment improved several types of stressors involved in inflammation and oxidative stress in HF diet-induced obese mice.

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AUTHOR DISCLOSURE STATEMENT

There are no conflicts of interest.

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