

Mentha cordifolia Leaf Extract Improves Hepatic Glucose and Lipid Metabolism in Obese Mice Fed with High-Fat Diet

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ABSTRACT: *Mentha cordifolia* (MC) is a popular herb used to flavor food in Thailand that exhibits several biological effects. The present study aimed to determine the role of MC in regulating glucose and lipid metabolism in mice fed a high-fat diet (HFD). ICR obese mice were fed an HFD (45 kcal% lard fat) for 12 weeks, with MC (100 and 200 mg/kg/d) treatment from Week 7. After treatment with MC for 6 weeks, mice showed significantly lower rates of hyperglycemia, hyperinsulinemia, hyperleptinemia, and hyperlipidemia, and increased amounts of serum adiponectin. Furthermore, in mice treated with MC, serum interleukin-6 and tumor necrosis factor alpha were significantly inhibited and liver histology results showed decreased lipid accumulation and liver triglyceride content vs. untreated mice. In addition, MC treatment was associated with smaller fat cells and lower gene expression of liver sterol regulatory element binding protein 1c, acetyl-CoA carboxylase, and fatty acid synthase. However, MC treatment was associated with higher carnitine palmitoyltransferase 1a gene expression and significantly higher rates of adenosine monophosphate-activated protein kinase (AMPK) phosphorylation in liver, but lower levels of phosphoenolpyruvate carboxykinase and glucose-6-phosphatase. These results indicate MC regulates glucose and lipid metabolism in a HFD-induced obese mouse model, possibly via activation of AMPK signaling pathway.

Keywords: insulin resistance, *Mentha cordifolia*, obesity

INTRODUCTION

The association between hepatic insulin resistance and development of non-alcoholic fatty liver disease (NAFLD) is marked by impaired glucose and lipid metabolism (Hardy et al., 2016). Obesity is a risk factor for both insulin resistance and NAFLD (Divella et al., 2019) and is associated with inflammatory pathway activation, which upregulates adipose tissue-derived pro-inflammatory cytokines including tumor necrosis factor alpha (TNF α) and interleukin-6 (IL-6) (Sharma et al., 2013). Key markers for predicting obesity, insulin resistance, and inflammation include secretion of adipose tissue cytokines, adiponectin, and leptin (Frühbeck et al., 2017).

Insulin resistance is associated with increased hepatic lipid deposition, up-regulation of sterol regulatory element binding protein 1c (SREBP1c), and depletion of free fatty acids (FFAs) β -oxidation (Ipsen et al., 2018).

SREBP1c is a key factor in fatty acid synthesis, activating lipogenic enzymes such as acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) (Ipsen et al., 2018). Obesity-related insulin resistance impacts hepatic lipogenesis by activating lipogenic genes (ACC and FAS) and suppressing fatty acid oxidation, including carnitine palmitoyltransferase 1a (CPT1a) gene expression (Naowaboot et al., 2016b; Tian et al., 2020).

In people with obesity-related insulin resistance, blood glucose levels may be elevated by increased liver gluconeogenesis. Phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) are key gluconeogenic enzymes activated in liver glucose production (Zhang et al., 2019), whereas adenosine monophosphate-activated protein kinase (AMPK) is the main regulator of lipogenesis, gluconeogenesis, and protein synthesis inhibition in liver (Jeon, 2016). AMPK activation is important for regulating lipid deposition in the liver since AMPK

Received 3 February 2021; Revised 18 March 2021; Accepted 22 March 2021; Published online 30 June 2021

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phosphorylation can inhibit SREBP-1c and downstream lipogenic enzymes (Jung et al., 2011; Fang et al., 2019).

Mentha cordifolia (MC) is a popular herb used to flavor Thai food and herbal tea. MC has been suggested to possess antioxidant and antihypertensive effects (Hardie, 2008) and to exhibit analgesic, anti-inflammatory, and antimutagenicity properties (Egawa et al., 2011). However, the role of MC in glucose and lipid metabolism impairment has not previously been reported. Here, obesity-related the biological activity of MC extracts was investigated in mice fed a high-fat diet (HFD) as a model for insulin resistance.

MATERIALS AND METHODS

Plant extraction

MC was obtained from Phatum Thani, Thailand. MC was identified by the Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand (voucher specimen code: SKP 095 13 03 01). Dried MC was extracted three times with distilled water at 100°C for 30 min, and aqueous extracts were filtered and freeze-dried. The dry powder had a yield of 14.76%.

Phytochemical screening of MC extracts

The bioactive components of MC extracts were determined using high-performance liquid chromatography with diode array detection and mass spectrometry (HPLC-DAD/MSD), as previously described (Duangjai et al., 2016). MC extracts was analyzed against phenolic standards. Semiquantitative data were analyzed by the peak area under the curve relative to the content of each component in the extract.

Animals and obesity induction

The animal experiment protocol was approved by the Animal Ethics Committee of Faculty of Medicine, Srinakharinwirot University, Bangkok, Thailand (AE: 7/2559). Thirty-two male ICR mice (National Laboratory Animal Center, Nakhon Pathom, Thailand) weighing 21~25 g were housed at 23~27°C with a 12-h light/dark cycle. Animals were fed a low-fat diet (LFD: D12450H, Research Diets Inc., Brunswick, NJ, USA) and water *ad libitum* for a week, followed by a LFD or HFD (D12451, Research Diets Inc.) for 12 weeks.

Experimental design

After 6-week on the LFD or HFD, mice were randomly divided into the following four groups (n=8 in each): a normal control group (NC) fed with LFD, an obese control group (OB) fed with HFD, and obese groups treated with MC at 100 and 200 mg/kg. MC was dissolved in distilled water, and the extract was administered orally

using intragastric tube daily for 6 weeks. Food intake and body weight were recorded every week. Fasting blood glucose (FBG) was checked after 6 weeks of treatment.

Sample collection

After 6 weeks of treatment, mice were fasted for 6 h then anesthetized with isoflurane inhalation. Blood samples were collected from the heart by cardiac puncture and the liver was removed and weighed. All samples were kept at -70°C until analysis.

Intraperitoneal glucose tolerance test (IPGTT)

After 5 weeks of treatment with MC, IPGTT was conducted in 6-h fasted mice as previously described (Naowaboot et al., 2016a). Mice were injected intraperitoneally with 2.0 g/kg of glucose, and blood glucose was measured at 0, 20, 60, and 120 min. The area under the curve (AUC) of blood glucose over the time was calculated using trapezoidal analysis.

Analysis of biochemical parameters in serum

Serum insulin, leptin, adiponectin, IL-6, and TNF α concentrations were estimated by enzyme-linked immunosorbent assay kits (Millipore Corporation, Billerica, MA, USA). Serum total cholesterol (TC), triglyceride (TG), and non-esterified fatty acid (NEFA) were evaluated by the enzymatic colorimetric kits (Wako Pure Chemical Industries, Ltd., Osaka, Japan).

Measurement of liver TG

Liver TG were extracted with isopropanol, and the supernatant was measured per the kit guidelines (Wako Pure Chemical Industries, Ltd.) (Oakes et al., 2001).

RNA extraction and quantitative real-time polymerase chain reaction (PCR)

Liver RNA was extracted using TRIzol[®] reagent (Invitrogen, Carlsbad, CA, USA) and reverse transcribed into cDNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Alameda, CA, USA). TaqMan-based quantitative real-time PCR (Applied Biosystems) was performed using a StepOnePlus[™] Real-time PCR System (Thermo Fisher Scientific, Waltham, MA, USA). TaqMan fluorogenic probes and primer sequences of SREBP1c, FAS, ACC, CPT1a, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were obtained from Applied Biosystems (Naowaboot et al., 2016b). Gene expression was expressed relative to the housekeeping GAPDH by using the formula $2^{-\Delta\Delta Ct}$.

Western blot

Liver protein (40 μ g) was separated using 12% Mini-PROTEAN[®] TGX precast gels (Bio-Rad Laboratories, Hercules, CA, USA). Western blot and band analysis

were performed as previously described (Naowaboot et al., 2016a). Immunoblotting was performed using primary antibodies for phosphorylated AMPK (pAMPK), total AMPK (tAMPK) (Millipore Corporation), G6Pase, PEPCK, and β -actin (Santa Cruz Biotechnology, Inc., Dallas, TX, USA). Membranes were then incubated with horseradish peroxidase conjugated secondary antibodies (Santa Cruz Biotechnology, Inc.). Protein bands were detected by the Clarity™ Western ECL substrate (Bio-Rad Laboratories), and images were obtained with an Odyssey Infrared Imaging System (LI-COR Biosciences, Lincoln, NE, USA). Band intensities were quantified by densitometry using a Gel-Pro™ Analyzer version 3.1 software (Media Cybernetics, Inc., Rockville, MD, USA). Proteins expression was normalized to expression of the housekeeping protein β -actin.

Histological analysis

Liver and epididymal fat tissue were placed in 10% formalin and processed for paraffin embedding. Sections of

3- μ m thickness were stained with hematoxylin and eosin (H&E), and the area of adipocytes was calculated (ImageJ Software, National Institutes of Health, Bethesda, MD, USA).

Statistical analysis

Data were expressed as mean \pm standard error of the mean (SEM). Data analysis was performed by one-way analysis of variance followed by Tukey's post-hoc test (SigmaStat 4.0, Systat Software, Inc., San Jose, CA, USA). $P < 0.05$ was considered statistically significant.

RESULTS

Phenolic compound content of MC

Phenolic compounds in MC extracts were analyzed by HPLC-DAD/MSD. The contents of gallic acid, protocatechuic acid, vanillic acid, caffeic acid, coumaric acid, ferulic acid, sinapic acid, catechin, rutin, and quercetin are shown in Table 1. The most abundant phenolic compounds in MC were caffeic acid (981.97 μ g/g), quercetin (410.99 μ g/g), and protocatechuic acid (270.25 μ g/g).

Effect of MC on metabolic parameters of an HFD-induced obese mouse model

Food intake in obese mice was significantly ($P < 0.05$) decreased compared with the normal control group. However, no significant differences were found between the obese control group and the obese group treated with MC. Indeed, energy intake did not significantly differ between all groups. Obese mice treated with MC showed a significant ($P < 0.05$) reduction in body weight compared with obese control mice, and was similar to mice in the

Table 1. Phenolic compound contents of *Mentha cordifolia* leaf extract (unit: μ g/g)

Phenolic compound	
Gallic acid	Not detected
Protocatechuic acid	270.25
Vanillic acid	107.04
Caffeic acid	981.97
Coumaric acid	25.55
Ferulic acid	47.57
Sinapic acid	2.83
Catechin	Not detected
Rutin	Not detected
Quercetin	410.99

Table 2. Effect of *Mentha cordifolia* leaf extract on metabolic parameters in HFD-induced obese mice

Parameters	NC	OB	OB + MC (mg/kg)	
			100	200
Body weight (g)	45.4 \pm 1.0	51.5 \pm 1.7*	47.0 \pm 1.0 [#]	46.9 \pm 0.9 [#]
Food intake (g/d/mouse)	4.7 \pm 0.1	4.0 \pm 0.1*	4.0 \pm 0.1*	4.0 \pm 0.1*
Energy intake (kcal/d/mouse)	17.5 \pm 0.2	18.4 \pm 0.4	18.8 \pm 0.3	18.6 \pm 0.4
Fasting blood glucose (mg/dL)	94.3 \pm 4.0	158.9 \pm 7.0*	108.7 \pm 6.1 [#]	97.0 \pm 1.8 [#]
Serum insulin (ng/mL)	1.1 \pm 0.2	4.0 \pm 0.6*	1.6 \pm 0.2 [#]	1.4 \pm 0.2 [#]
Serum leptin (ng/mL)	5.2 \pm 1.4	19.8 \pm 2.7*	6.1 \pm 1.7 [#]	5.6 \pm 1.1 [#]
Serum adiponectin (μ g/mL)	9.0 \pm 0.4	5.7 \pm 0.4*	7.0 \pm 0.2 [#]	7.1 \pm 0.3 [#]
Serum TNF α (pg/mL)	4.0 \pm 0.7	15.6 \pm 1.6*	6.4 \pm 0.9 [#]	4.5 \pm 0.8 [#]
Serum IL-6 (pg/mL)	9.0 \pm 1.3	27.0 \pm 4.6*	13.6 \pm 0.6 [#]	13.2 \pm 0.6 [#]
Serum TC (mg/dL)	95.8 \pm 4.3	200.4 \pm 7.7*	156.4 \pm 9.6 [#]	155.7 \pm 9.4 [#]
Serum TG (mg/dL)	78.7 \pm 9.4	154.0 \pm 6.3*	91.7 \pm 10.7 [#]	94.8 \pm 4.1 [#]
Serum NEFA (mEq/L)	1.0 \pm 0.1	1.8 \pm 0.1*	1.2 \pm 0.1 [#]	1.1 \pm 0.1 [#]

Values are mean \pm SEM (n=8 per group).

Significant differences were assessed using one-way ANOVA and Tukey's post hoc test, * $P < 0.05$ vs. normal control group and [#] $P < 0.05$ vs. obese control group.

NC, normal control group; OB, obese control group; OB + MC, obese groups treated with *Mentha cordifolia* at 100 and 200 mg/kg; TNF α , tumor necrosis factor alpha; IL-6, interleukin-6; TC, total cholesterol; TG, triglyceride; NEFA, non-esterified fatty acid.

normal control group (Table 2).

Prior to initiating treatment with MC, FBG was measured on Week 6 of HFD feeding to establish baseline values. Average baseline FBG levels were 87 mg/dL, 135 mg/dL, 134 mg/dL, and 131 mg/dL for the NC, OB, obese with MC 100 mg/kg, and obese with MC 200 mg/kg groups, respectively (data not shown). After 6 weeks of treatment with MC, FBG of the obese control mice was significantly higher than the normal mice. Furthermore, FBG of obese mice treated with 100 or 200 mg/kg MC were significantly lower than FBG of obese control mice ($P<0.05$) (Table 2). Indeed, obese mice treated with MC 200 mg/kg had FBG levels almost as low that of normal control mice.

High levels of serum insulin observed in obese mice were significantly ($P<0.05$) decreased by treatment with MC (Table 2). Furthermore, MC treatment significantly ($P<0.05$) decreased the elevated levels of serum leptin, IL-6, and TNF α in obese mice (Table 2). In addition, obese mice treated with MC groups had higher levels of serum adiponectin compared with the non-treated obese group ($P<0.05$) (Table 2). After treatment with MC for 6 weeks, serum concentrations of TC, TG, and NEFA were significantly ($P<0.05$) reduced compared with those of obese control mice (Table 2).

In glucose tolerance tests, mice treated with MC exhibited significantly ($P<0.05$) higher inhibition of high blood glucose at time intervals of 60 and 120 min compared with obese control mice (Fig. 1A). Indeed, the AUC for the MC-treated groups were significantly lower than the AUC for the obese control group (Fig. 1B).

Effect of MC on histological changes in epididymal fat of an HFD-induced obese mouse model

The ratio of weight of epididymal fat to body weight was significantly ($P<0.05$) less in obese mice treated with MC compared with the obese control group (Fig. 2A).

Furthermore, MC treatment significantly ($P<0.05$) reduced the sizes of enlarged fat cells (Fig. 2B) compared with those of obese control mice. These results were correlated with H&E staining of fat cells, as shown in Fig. 2C.

Effect of MC on histological changes in liver of an HFD-induced obese mouse model

Obese mice treated with MC showed significant decreases in the ratio of liver weight to body weight and liver TG deposition compared with obese control mice (Fig. 3A and 3B). In addition, liver histology results showed that obese mice treated with MC had fewer lipid depositions than obese control mice (Fig. 3C).

Effect of MC on gene and protein expression in liver of an HFD-induced obese mouse model

Treatment with MC significantly ($P<0.05$) suppressed SREBP1c, FAS, and ACC gene expression compared in obese mice (Fig. 4A, 4B, and 4C, respectively). However, obese mice treated with MC had significantly ($P<0.05$) increased expression of CPT1a compared with obese control mice (Fig. 4D).

The elevated protein expression of PEPCK (Fig. 5A) and G6Pase (Fig. 5B) observed in obese control mice was significantly ($P<0.05$) reduced by treatment with 100 or 200 mg/kg MC (Fig. 5). Interestingly, expression of phosphorylated AMPK was significantly ($P<0.05$) increased in obese mice treated with MC groups compared with obese control mice (Fig. 5C).

DISCUSSION

This study demonstrated that MC extracts can improve the impaired glucose and lipid metabolism observed in a mouse model of HFD-induced obesity. MC extracts ef-

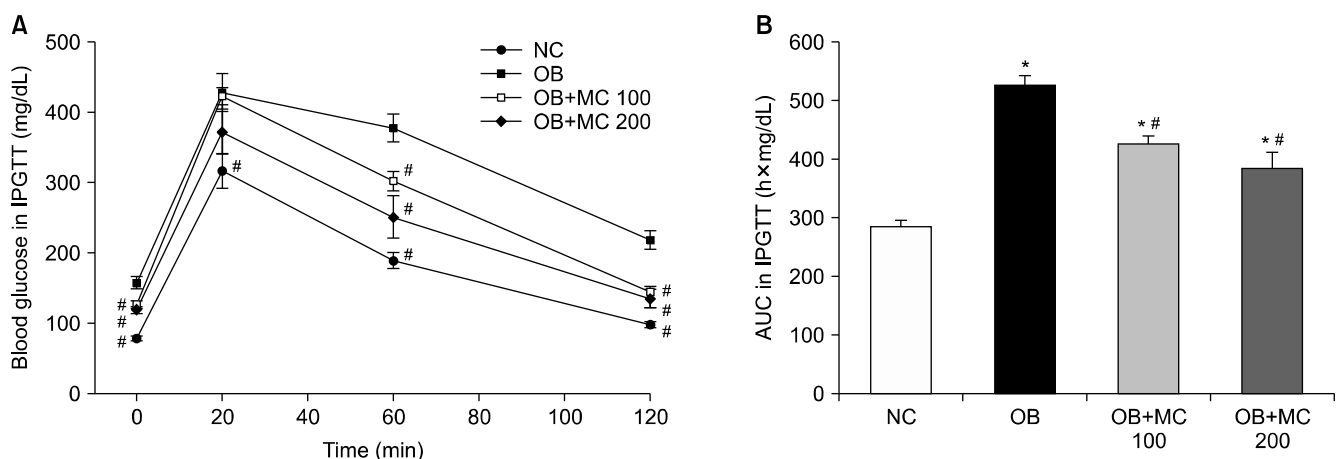


Fig. 1. Effect of *Mentha cordifolia* leaf extracts on (A) IPGTT and (B) AUC in high-fat diet-induced obese mice. Data are mean \pm SEM (n=8). * $P<0.05$ vs. normal control group and # $P<0.05$ vs. obese control group. IPGTT, intraperitoneal glucose tolerance test; AUC, the area under the curve; NC, normal control mice; OB, obese mice; MC, *Mentha cordifolia* leaf extract.

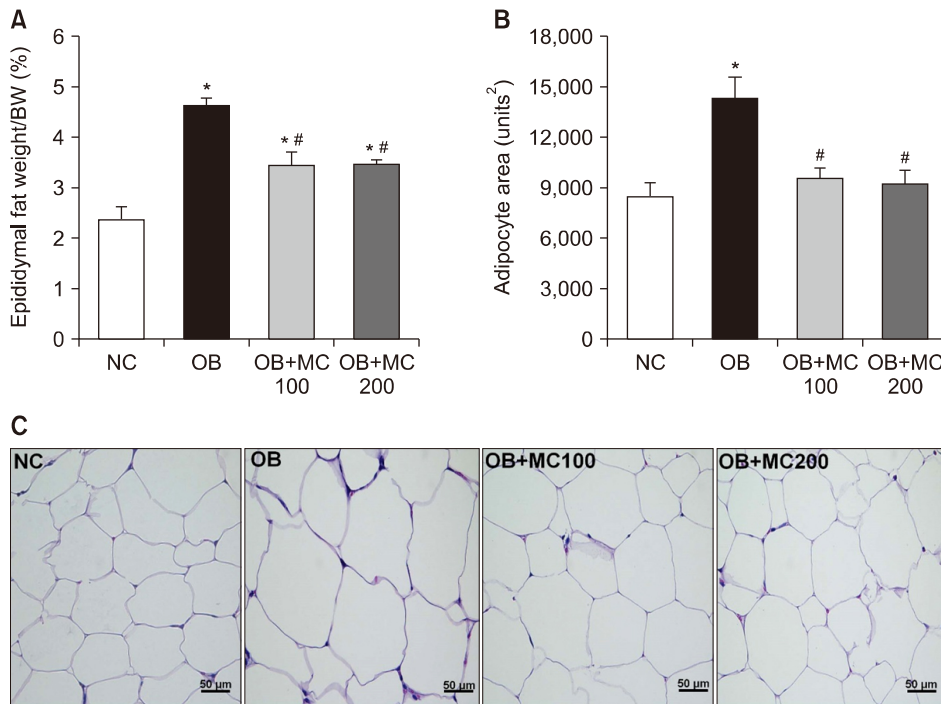


Fig. 2. Effect of *Mentha cordifolia* leaf extract on (A) epididymal fat weight, (B) fat cell size, and (C) epididymal fat histological examination (hematoxylin and eosin staining, 400X) in high-fat diet-induced obese mice. The obese groups treated with MC showed smaller fat cells than the obese control group. Data are mean \pm SEM (n=8). * P <0.05 vs. normal control group and # P <0.05 vs. obese control group. BW, body weight; NC, normal control mice; MC, *Mentha cordifolia* leaf extract.

fectively targeted insulin resistance in this mouse model, and improved hyperglycemia and hyperlipidemia.

After 6 weeks of MC treatment, body weight of mice in the treated groups was only slightly increased compared with mice in the normal control group. However, MC treatment significantly decreased body weight compared with normal obese mice. These results suggest that MC extracts may have a role in regulating metabolic change in obese conditions.

Hyperglycemia has been shown to be present in HFD-induced obesity-related insulin resistant animal models (Naowaboot et al., 2018; Liu et al., 2019; Thapa et al., 2019). Our study demonstrated that MC extracts significantly decrease hyperglycemia, glucose intolerance, and serum insulin in this mouse model. Leptin, an adipose tissue-derived cytokine, acts as a regulator of appetite and energy homeostasis. The association between leptin dysfunction and progression of insulin resistance has previously documented (van der Wijden et al., 2015), and hyperleptinemia has been associated with insulin resistance in HFD-induced obese mice (Nguyen et al., 2019). After 6 weeks of treatment with MC, obese mice had decreased hyperleptinemia and significantly elevated levels of serum adiponectin. Adiponectin is responsible for maintaining insulin sensitivity, with decreased level of adiponectin leading to obesity-related insulin resistance (Forný-Germano et al., 2019). Moreover, studies have shown that administration of adiponectin can improve insulin sensitivity (Achari and Jain, 2017). Therefore, the results of the present study suggest that MC extracts may have a role in managing glucose metabolism and insulin sensitivity in obesity-related insulin resistance.

The liver is a major organ involved in decreasing glucose production and restoring glucose metabolism in diabetic patients (Lam, 2016), with activation of PEPCK and G6Pase being crucial for increasing liver glucose production (Sharabi et al., 2015). In individuals with insulin resistance, increases in hepatic glucose production activates gluconeogenesis and glycogen pathways (Jayanthi and Subramanian, 2015). In this study, treatment with MC extracts decreased protein expression of PEPCK and G6Pase in the liver. In addition, phosphorylated AMPK, which is involved in controlling glucose and lipid metabolism (Steinberg and Carling, 2019), was increased in the liver of obese mice treated with MC. Therefore, MC-activated AMPK may help suppress gluconeogenesis in HFD-induced obesity-related insulin resistance.

Activation of AMPK is key for regulating lipid metabolism (Steinberg and Carling, 2019) by reducing lipid content (Esquejo et al., 2018). Our study showed that treatment with MC extracts for 6 weeks significantly decreased serum lipid profiles, TC, TG, and NEFA. The decreased levels of TG in liver were correlated with lipid deposition in MC-treated groups, as observed in histology studies, and MC treatment decreased liver weight in obese mice. Stimulation of AMPK activity can inhibit SREBP1c-mediated lipogenesis (Li et al., 2011; Smith et al., 2016). In the present study, obese mice treated with MC extracts had lower expression of the transcription factor SREBP1c and lipogenic enzymes ACC and FAS in liver than obese control mice. Moreover, MC extracts may increase CPT1a gene expression in liver. This study indicates that activation of AMPK by MC extracts could inhibit hepatic lipogenesis, therefore MC extracts may improve impaired

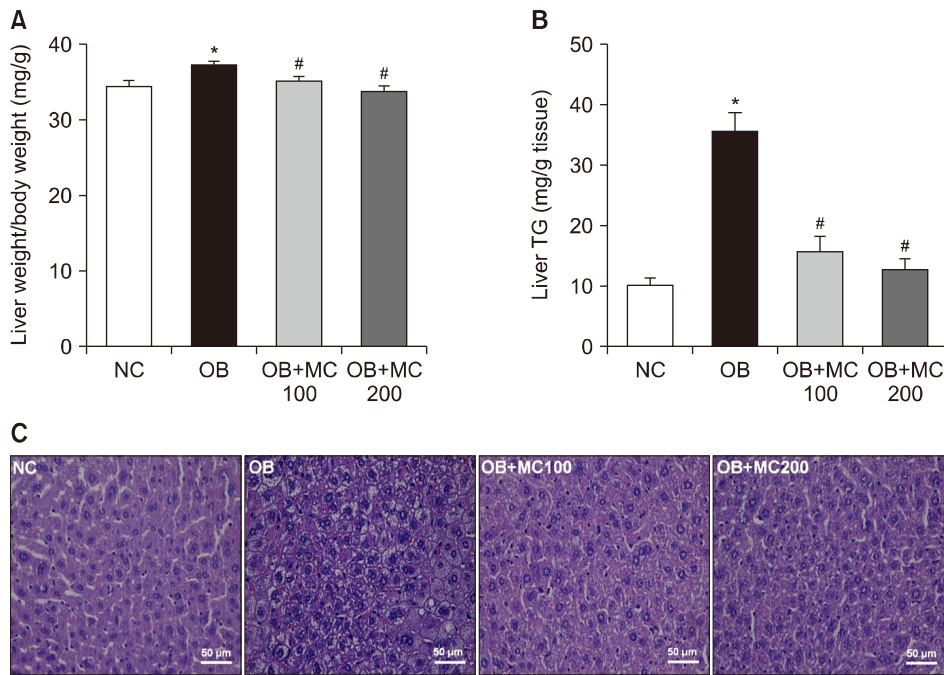


Fig. 3. Effect of *Mentha cordifolia* leaf extract on (A) liver weight, (B) liver TG, and (C) liver histology (hematoxylin and eosin staining, 400X) in high-fat diet-induced obese mice. The liver histology results showed that lipid accumulation in obese groups treated with MC was lower than obese control groups. Data are mean±SEM (n=8). * $P < 0.05$ vs. normal control group and # $P < 0.05$ vs. obese control group. TG, triglyceride; NC, normal control mice; OB, obese mice; MC, *Mentha cordifolia* leaf extract.

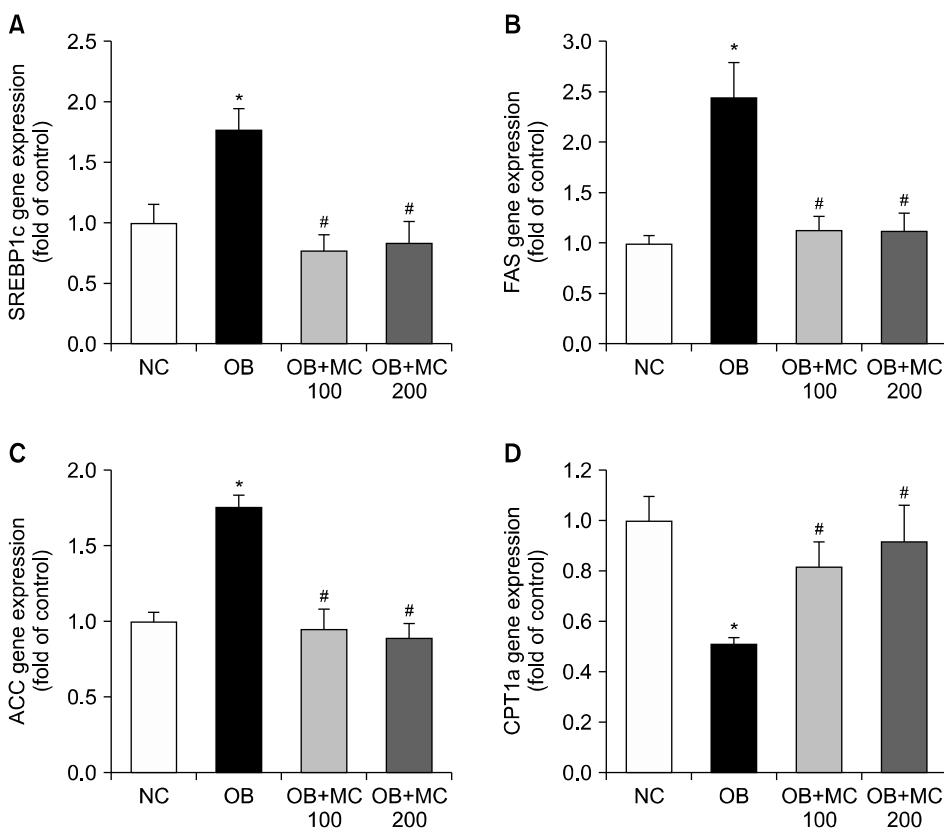


Fig. 4. Effect of *Mentha cordifolia* leaf extract on liver (A) SREBP1c, (B) FAS, (C) ACC, and (D) CPT1a gene expression in high-fat diet-induced obese mice. Data are mean±SEM (n=8). * $P < 0.05$ vs. normal control group. # $P < 0.05$ vs. obese control group. SREBP1c, sterol regulatory element binding protein 1c; FAS, free fatty acid; ACC, acetyl-CoA carboxylase; CPT1a, carnitine palmitoyltransferase 1a; NC, normal control mice; OB, obese mice; MC, *Mentha cordifolia* leaf extract.

lipid metabolism via activating AMPK.

White adipose tissue is the primary site for inducing inflammation in obesity, following which inflammation can spread to other tissues such as liver and skeletal muscle to result in low-grade systemic inflammation (Zatterale et al., 2020). In a previous study, hyperlipidemia was shown to be related to increased FFA released by white adipose tissues (van Dam et al., 2017). Higher

amounts of lipid are able to be deposited in organs other than adipose tissue (such as liver), which can activate low-grade inflammation and insulin resistance in those organs (Gross et al., 2017). Treatment with MC significantly reduced the weight of epididymal adipose tissue in obese mice, and decreased the sizes of enlarged fat cells, as shown via histology examination. Previous studies have reported a relationship between hypertrophic

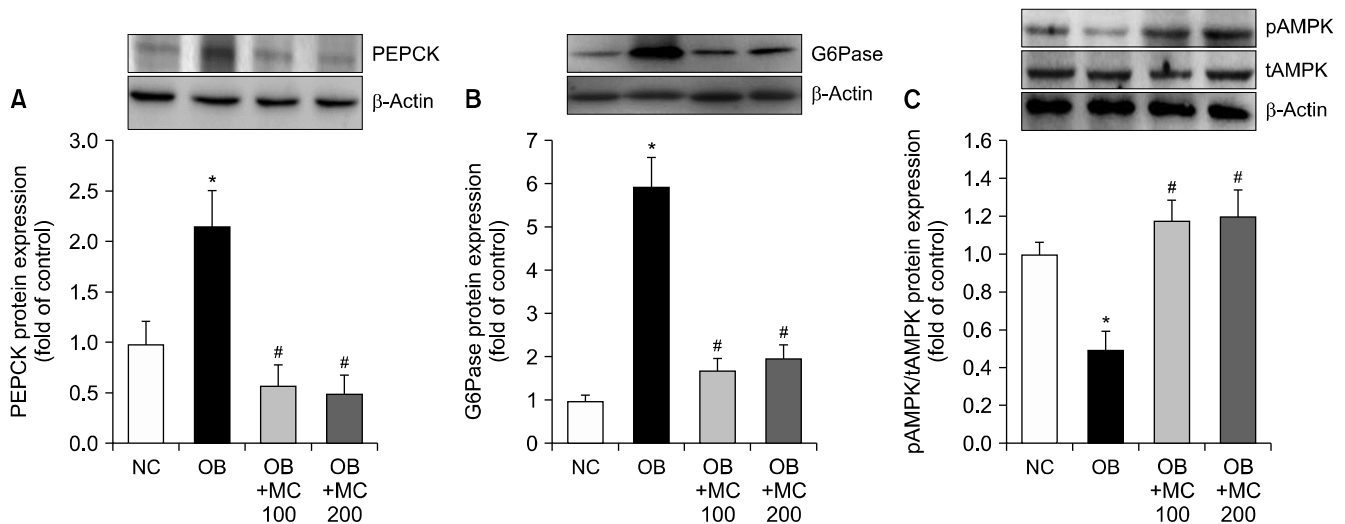


Fig. 5. Effect of *Mentha cordifolia* leaf extract on hepatic protein expression of (A) PEPCK, (B) G6Pase, and (C) pAMPK in high-fat diet-induced obese mice. Data are mean \pm SEM (n=8). * P <0.05 vs. normal control group and # P <0.05 vs. obese control group. PEPCK, phosphoenolpyruvate carboxykinase; G6Pase, glucose-6-phosphatase; pAMPK, phosphorylated adenosine monophosphate-activated protein kinase; tAMPK, total AMPK; NC, normal control mice; OB, obese mice; MC, *Mentha cordifolia* leaf extract.

adipocytes and insulin resistance (Longo et al., 2019) and a correlation between hypertrophic adipocytes and increased release of pro-inflammatory cytokines such as TNF α and IL-6 (Rodríguez et al., 2015). In the present study, increased serum TNF α and IL-6 in obese mice was reduced by treatment with MC extracts. Depletion of pro-inflammatory cytokines following MC treatment may be related to the smaller sizes of the enlarged fat cells. Activation of AMPK has a significant role in alleviating various conditions such as inflammatory diseases, along with cardiovascular and metabolic disorders (Hardie et al., 2012), and has been reported to suppress inflammatory signaling pathways. In addition, activated AMPK acts as an inhibitor of TNF α , IL-6, and IL-1 β production (Mancini et al., 2017). These findings indicate that inhibition of inflammation by MC extracts is correlated with stimulation of AMPK activity.

In this study, we described the phenolic compound profile of MC extracts. The major phenolics in MC extracts were caffeic acid, quercetin, and protocatechuic acid (981.97, 410.99, and 270.25 μ g/g, respectively), all of which have been reported to be stable in water heated up to 150°C (Cheng et al., 2014; Sharma et al., 2015). Studies have shown that caffeic acid exhibits antidiabetic activity by stimulating AMPK in L6 myocytes (Eid et al., 2017), enhancing glucose uptake, decreasing inflammatory cytokine, and suppressing G6Pase gene in mice and HepG2 cells (Nie et al., 2017). Furthermore, quercetin helps improve insulin sensitivity by activating insulin- and AMPK-dependent pathways in L6 myocytes (Jiang et al., 2019), and protocatechuic acid helps alleviate insulin resistance in visceral fat of obese subjects (Ormazabal et al., 2018). Therefore, the major phenolic compounds present in MC extracts may be associated with improved in-

ulin function in obesity-related insulin resistance.

The present study used a mouse model to investigate the effects of MC on hepatic glucose state and lipid metabolism impairment. MC at a dose of 200 mg/kg/d was shown to be effective for improving glucose and lipid metabolism in mice. In order to assess the possibility of applying MC at this dosage to human, we performed a dose conversion, using the body surface area normalization method approved by the U.S Food and Drug Administration (Hosseini et al., 2018). In this method, the human equivalent dose (mg/kg) is determined to be equal to the animal dose (mg/kg) multiplied by the ratio of animal K_m to human K_m , whereby K_m is a correction factor estimated by dividing average body weight to body surface area. From this calculation, the daily dose for human was calculated to be 16.216 mg/kg [972.96 mg (approximately 1 g) for adults with the body weight of 60 kg]. As mice treated with MC 200 mg/kg/d did not show any abnormalities or side effects from feeding (e.g., diarrhea), the human equivalent dose may be safe to use. However, further studies are needed to clarify the safety of MC as an alternative agent in human.

This study shows that MC extracts may be useful agents for restoring impaired metabolism of glucose and lipids in HFD-induced obesity-related insulin resistance. Indeed, MC extracts may improve glucose and lipid metabolism by reducing hyperglycemia, hyperinsulinemia, hyperleptinemia, hyperlipidemia, hepatic lipid accumulation and levels of inflammatory cytokines. The mechanism for these effects may be strongly associated with AMPK activation. Therefore, MC extract may be a potential therapeutic agent for improving the impaired glucose and lipid metabolism present in obesity-related insulin resistance.

ACKNOWLEDGMENTS

This study was supported by Faculty of Medicine Research Fund, Thammasat University (Contract No. 2-14/2560) and Thammasat University Research Fund (Contract No. TUFT022/2563). We thank Dr. Benjaporn Buranrat for providing the opportunity for collecting this medicinal plant.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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