J Korean Soc Appl Biol Chem (2015) 58(5):643–649
DOI 10.1007/s13765-015-0087-x

Online ISSN 2234-344X
Print ISSN 1738-2203

ARTICLE

Preventive responses to a standardized extract of *Houttuynia cordata* supplemented diet in obesity induced by a high-fat diet in mice

Hee-Sook Park[1,2] · **Soon-Mi Shim**[2]

Received: 26 March 2015 / Accepted: 20 May 2015 / Published online: 30 May 2015

Abstract The current study investigated preventive responses to *Houttuynia cordata* (HC) standardized extract in obesity induced by a high-fat diet (HFD) in mice. High-performance liquid chromatography was carried out to analyze bioactive components in HC. Mice were divided into HFD groups as a control and HFD groups fed with 1 and 4 % of HC supplementation diet. Changes of the body weight of mice, blood biological factors, and histopathological markers were measured. Bioactive components from HC were characterized to contain chlorogenic acid, caffeic acid, rutin, and quercitrin. HC supplementation reduced body weight, glucose, and total cholesterol levels, but it did not affect aspartic acid transaminase, alanine transaminase, or triglyceride amounts in plasma from mice induced by HFD. A 4 % HC supplementation showed a remarkable decrease in the number of large adipocyte in subcutaneous and abdominal adipose tissue. Histopathological evaluation of liver injury induced by HFD was observed to be improved by HC supplementation, leading to attenuated levels of cytoplasmic vacuolation of hepatocytes. From these results, we explored that standardized extract of HC could be a potential natural product to be directly associated with the control of weight.

✉ Soon-Mi Shim
soonmishim@sejong.ac.kr

[1] Research Group of Nutraceuticals for Metabolic Syndrome, Korea Food Research Institute, Seoul, Gyeonggi-do 463-736, Republic of Korea

[2] Department of Food Science and Technology, Sejong University, 98 Gunja-dong, Gwangjin-gu, Seoul 143-747, Republic of Korea

Keywords Adipocyte · High-fat diet · Liver injury · Standardized extract of *Houttuynia cordata*

Introduction

Houttuynia cordata (HC) Thunb (Saururaceae), which is called E-sung-cho in Korea, is a perennial herb that is native to Korea, Japan, southern China, and south-east Asia. The root, young shoots, leaves, and sometimes the whole plant are traditionally used to prevent various human diseases. It has been reported that *H. cordata* Thunb provided many biological properties such as anti-mutagenic (Chen et al. 2003), antioxidant (Kusirisin et al. 2009), anti-allergic (Han et al. 2009), antiviral (Hayashi et al. 1995), and antibacterial activities (Lu et al. 2006)). It contains many flavonoids (quercitrin, isoquercitrin, and rutin) (Meng et al. 2005), alkaloids (aristolactam B, norcepharadione B, and splendidine) (Kim et al. 2001), and volatile components of essential oils (methyl-n-nonyl ketone, lauraldehyde, and β-myrcene) (Xu et al. 2005).

Obesity correlates positively with development of cardiovascular diseases, type 2 diabetes mellitus, osteoarthritis, and certain forms of cancer (Aronne and Isoldi 2007). It causes prolonged energy imbalance and increases the amount of triglyceride (TG) content in adipose tissue (Van Herpen and Schrauwen-Hinderling 2008). Accumulation of excess lipids in non-adipose tissues has deleterious consequences on organ function through resultant cell dysfunction or cell death (Unger and Orci 2001; Schaffer 2003). Lipotoxicity is known as cellular toxicity mediated by lipids, implicating the pathophysiology of metabolic syndrome (Slawik and Vidal-Puig 2006; Unger et al. 2010). The physiological mechanisms of lipotoxicity are involved in contributing the excess of energy derived from overnutrition (Schaffer 2003).

Polyphenols, naturally occurring phytochemicals such as catechins, anthocyanines, resveratrol, and curcumin are involved in energy metabolism, adiposity, and obesity (Aggarwal 2010). Clinical and epidemiological studies have also demonstrated that polyphenols have an important role in anti-inflammatory and anti-obesity properties (Hsu and Yen 2007, 2008). Current data suggest that polyphenols may have the potential to modulate mitochondrial function, consequently preventing adipose dysfunction in obesity. For example, chlorogenic acids are known to quench oxygen radical formation and to inhibit the formation of inflammatory mediators. Thus, chlorogenic acid plays an active role in the protective mechanism against inflammatory response (Xu et al. 2010). Zhang et al. (2011) investigated that oral administrations of chlorogenic acid decreased triglyceride in the plasma, liver, and skeletal muscles in anima. Rutin reduced oxidative stress and glycerol-3-phosphate dehydrogenase (GPDH) activity in rats with obesity induced by a high-fat diet.

Since centuries, medicinal plants have been used for treating liver diseases. As potential hepatoprotective agent, several plant sources have diverse chemical structures (Negi et al. 2008). Yang et al. (2014) showed that antioxidant of standardized extract inhibited heavy metal-induced oxidative stress on cell system. Various medicinal plants, contained with chlorogenic acid, have been hepatoprotective effect against liver damage agent (Koriem and Soliman 2014; Xu et al. 2014; Ji et al. 2013). The aim of this study was to examine whether *H. cordata* ethanolic extract is effective in prevention of obesity and protection of liver damage induced by a high-fat diet.

Materials and methods

Preparation of *H. cordata* extract

HC was harvested from Seongju-gun, Gyeongbuk, Korea in November, 2011. HC was washed with deionized water and then, it was lyophilized and crushed into powder. Freeze-dried HC powder was extracted twice in 50 % ethanol at 100 °C for 6 h and filtered. The ethanolic extract was evaporated under vacuum conditions at 50 °C.

Analysis of bioactive components in *H. cordata* ethanol extract

An Agilent 1200 series high-performance liquid chromatography (HPLC) system equipped with a diode array detector was carried out for quantification and identification of bioactive components from 50 % extract of *H. cordata.* A Zorbax XDB-C18 column (4.6 × 50 mm, 1.8 μm) was used with a flow rate of 0.7 mL/min at room temperature. Mobile phases consisted of 0.1 % formic acid in water (phase A) and acetonitrile (phase B, v/v) were used. The linear gradient mobile phase program was used as follows: 0–3 min, 5 % B; 3–5 min, 5–10 % B; 5–10 min, 10–20 % B; 10–25 min, 20–40 % B; 25–35 min, 40–90 % B, followed by 5 min of re-equilibration. The injection volume was 5 μL for each sample. The wavelength of the UV detector was set at 280 and 360 nm. Chromatographic peaks in samples were identified as comparing retention times of pure standards (chlorogenic acid, caffeic acid, rutin, and quercitrin). Quantitative analysis was conducted using a standard curve.

Animal experiment

Three-week-old male ICR mice were obtained from OrientBio (Korea) and housed in a controlled environment (22 ± 2 °C, 12 h light–dark cycle). After acclimation for a week, six mice were randomly assigned to one of the four treatment groups. Mice in a normal diet group (ND, 5L79, OrientBio) were fed a standard chow diet during the entire experiment period (13 weeks). Mice in high-fat diet (HFD) groups were fed a diet containing 60 % of calories derived from fat (Research Diet, USA) for 7 weeks. After feeding the mice HFD for 6 weeks, 1 and 4 % of *H. cordata* extract mixed with HFD were fed to the HFD + 1 % HC and HFD + 4 % HC groups, respectively for 7 weeks. All animals had free access to water, and the average group body weight and food intakes were recorded every week. At the end of the experiment (13 weeks), mice were sacrificed after an overnight fasting. Subcutaneous and abdominal adipose tissue and liver were collected for analysis and frozen in −80 °C.

Blood and histopathological analysis

Blood samples were drawn from the abdominal aorta into a vacuum tube and allowed to stand at room temperature. Blood was immediately mixed with EDTA, and plasma was isolated by centrifugation at 3000 rpm for 10 min. Plasma levels of glucose were analyzed by Accuchek (Roche, Schweiz). Plasma TG was assayed by using commercial kits (Sigma-aldrich, USA). For the analysis of biochemical factors, Aspartic acid transaminase (AST), alanine transaminase (ALT), high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol were analyzed by auto-analyzer (BPC Biosed, Italy). For the histological analysis, the abdominal adipose tissues were fixed with 10 % paraformaldehyde, dehydrated in a graded alcohol series, and embedded in paraffin wax using an automated tissue processor. Five micrometer sections were cut and stained with Oil red O solution. Images were acquired by microscopy (Olympus CKX41, Olympus Co. Ltd., Japan).

Measurement of plasma adiponectin level

Plasma concentration of adiponectin was measured using enzyme-linked immunosorbent assay kits (R&D system, USA) according to the instruction manual.

Statistical analysis

Results were expressed as mean ± standard deviation from at least three independent experiments. Statistical differences among groups were evaluated by one-way analysis of variance (ANOVA) by using SPSS 12.0. (SPSS, Inc., USA).

Results and discussion

Identification and quantification of bioactive components in HC ethanol extract

Six peaks were detected by UV spectrum at both 260 and 380 nm of 50 % ethanol extract HC (Fig. 1). According to relative retention time as of each standard, chlorogenic acid, caffeic acid, rutin, and quercitrin hydrate were detected. Amounts of 27.35 ± 0.35, 17.73 ± 0.40, 46.13 ± 0.26, and 77.69 ± 0.28 μg/kg of fresh weight were quantified for chlorogenic acid, caffeic acid, rutin, and quercitrin hydrate, respectively. Previous studies chemically identified bioactive components in HC extract such as quercitrin, isoquercitrin, rutin, hyperoside, chlorogenic acid, quercetin (Xu et al. 2006; Chou et al. 2009; Meng et al. 2009; Jang et al. 2011). Results from the current study showed a similar profile of bioactive components in HC extract to those from previous studies. Cho et al. (2010) found that chlorogenic acid significantly lowered body weight, and triglyceride and cholesterol contents. Several studies have been shown that supplementation of chlorogenic acid in the high-fat diet did not reduce body weight but has impaired fatty acid oxidation by increasing lipid content and steatosis (Mubarak et al. 2013). Thus, we further investigated the effect of HC standardized extract containing chlorogenic acid as well as other flavonoids on anti-obesity properties and improve lipid metabolism in high-fat diet-induced-obese mice.

Effect of HC supplementation on body weight in HFD-fed mice

HFD-fed mice gradually increased in body weight for 13 weeks compared to the NFD-fed mice (Fig. 2A). Body weight gain by induced HFD was extensively suppressed by HC supplementation to mice from 8 weeks, indicating that 4 % HC was more effective on body weight reduction than 1 % HC. At the 12 weeks, supplementation of HC was significantly decreased body weight compared to HFD diet (Fig. 2B). Similar to the current study, Miyata et al. showed decreasing pattern for body weight of mice by HC water extract (Mubarak et al. 2013). Additionally, food intake of HC showed decreasing pattern compared to HFD without significant difference (Fig. 2C). Thus, it would be suppression of body weight due to decreasing of food intake.

Effect of HC supplementation on biochemical analysis of plasma

Plasma obtained from HFD-fed mice for 6 weeks after 7 weeks of 1 or 4 % HC supplementation was analyzed for biochemical factors (Table 1). The fasting blood glucose levels were found to be significantly lower in HFD-fed mice with 1 and 4 % of HC than the NFD or HFD group ($p < 0.05$), decreasing to 60 and 56.6 % of glucose levels of HFD-fed mice, respectively. For serum parameters of liver injury, AST from mice fed HFD + 1 % HC and HFD + 4 % HC group increased 2.0 and 2.7 times more than that of HFD group, respectively. In contrast to AST, ALT level was not significantly different among groups. Total cholesterol levels were not significantly different in all groups. For serum TG levels, 1 and 4 % HC extracts were not effective on decreasing TG levels when compared to NFD-fed mice. Results from the study are inconsistent with results from previous studies. For instance, it was found that absorption of bioactive components in HC water extract was faster than TG in the small intestine, indicating plasma TG levels decreased by HC water extract supplementation diet (Mubarak et al. 2013). Lin et al. (2013) also found that HC water extract in mice consuming a high saturated fat diet decreased triglyceride and total cholesterol contents in plasma from HFD-treated mice. This discrepancy may likely be due to the fact that area under the curve of the plasma TG during HC supplementation was not assessed in the current study. Furthermore, there are differences in the profiles of bioactive components, which could hinder the time of the peak of plasma TG level between water extract and ethanol extract, suggesting that bioactive components in HC water extract could be more effective on plasma TG level than those in ethanol extract of HC.

Effect of HC supplementation on histopathological analyses in fat tissue

Histological slices of fat samples with the subcutaneous and abdominal adipose tissue after HFD followed by HC supplementation were analyzed (Fig. 3). In the case of subcutaneous fat tissue from mice with 4 % HC

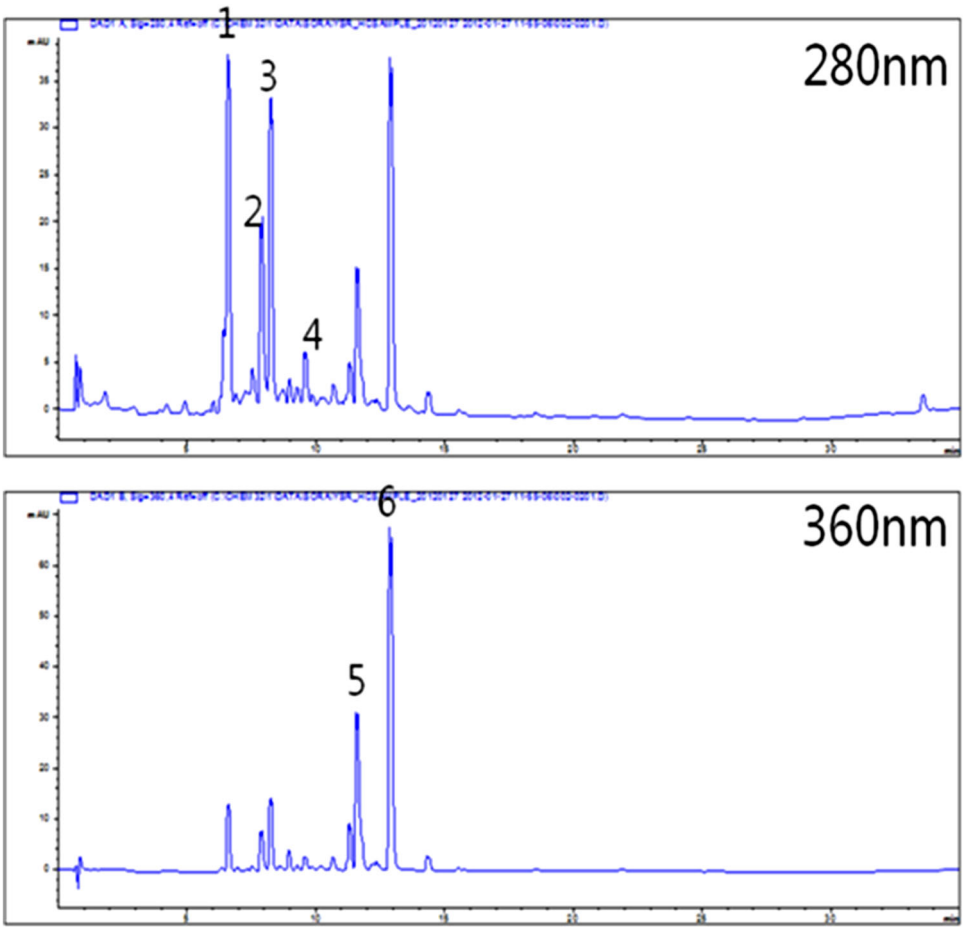

Fig. 1 Chromatogram of bioactive components in 50 % of ethanol extract of *Houttuynia cordata* (HC). *Peak 1* unknown, *Peak 2* chlorogenic acid, *Peak 3* caffeic acid, *Peak 4* unknown, *Peak 5* rutin, *Peak 6* quercitrin hydrate

supplementation, the greatest changes in number of small and large adipocytes were increased and decreased, respectively (Fig. 3A). Similar results were also obtained in abdominal fat tissue. HFD-fed mice with 4 % HC in the study showed a decreasing pattern in abdominal adipocyte sizes compared to that from NFD and HFD groups (Fig. 3B). These results suggest that HC exerts anti-obesity effects on adipose tissue and imply that vessel function would be improved by HC administration.

Effect of HC supplementation on histological analyses in liver injury induced by HFD

Microscopic views of the liver of mice are shown in Fig. 4. It was revealed that cytoplasmic vacuolation of hepatocytes which is around the central vein was more apparent in HFD-fed mice than in NFD-fed mice. In mice with HC supplementation diets, a markedly low level of cytoplasmic vacuolation of hepatocytes around the portal vein was observed compared with that in cells from HFD-fed mice. In contrast to biochemical analysis for liver injury, hepatic fat deposition and associated injury were found by histopathological evaluation in the high-fat diet group (Fig. 4). Oral administration of HC extract to HFD mice for seven weeks markedly reversed histopathologic changes without a significant difference from the control group. The result indicated that HC extract could effectively suppress HFD-induced lipotoxic liver injury. Supplementation of HC water extract to HFD by drinking water reduced the hepatic level of saturated fatty acids, suggesting suppression of HFD-induced oxidative and inflammatory stress in the liver (Lin et al. 2013). In the study, chlorogenic acid, caffeic acid, rutin, and quercitrin hydrate were identified as bioactive components in HC ethanol extract. There was a significant difference in body weight gains between the HFD-fed mice with and without a HC supplemented diet. Blood biochemical factors such as TG, total cholesterol, and ALT in plasma elevated by HFD were not significantly improved by HC ethanol extract supplementation. However, the levels of glucose and AST in plasma from HFD with 1 and 4 % HC supplementation were reduced. The current study found that HC supplementation decreased the size of adipocytes from subcutaneous and abdominal fat tissue in mice fed a HFD without affecting triglyceride content in the blood. Histopathological analysis of liver injury induced by HFD exhibited considerable reduction in

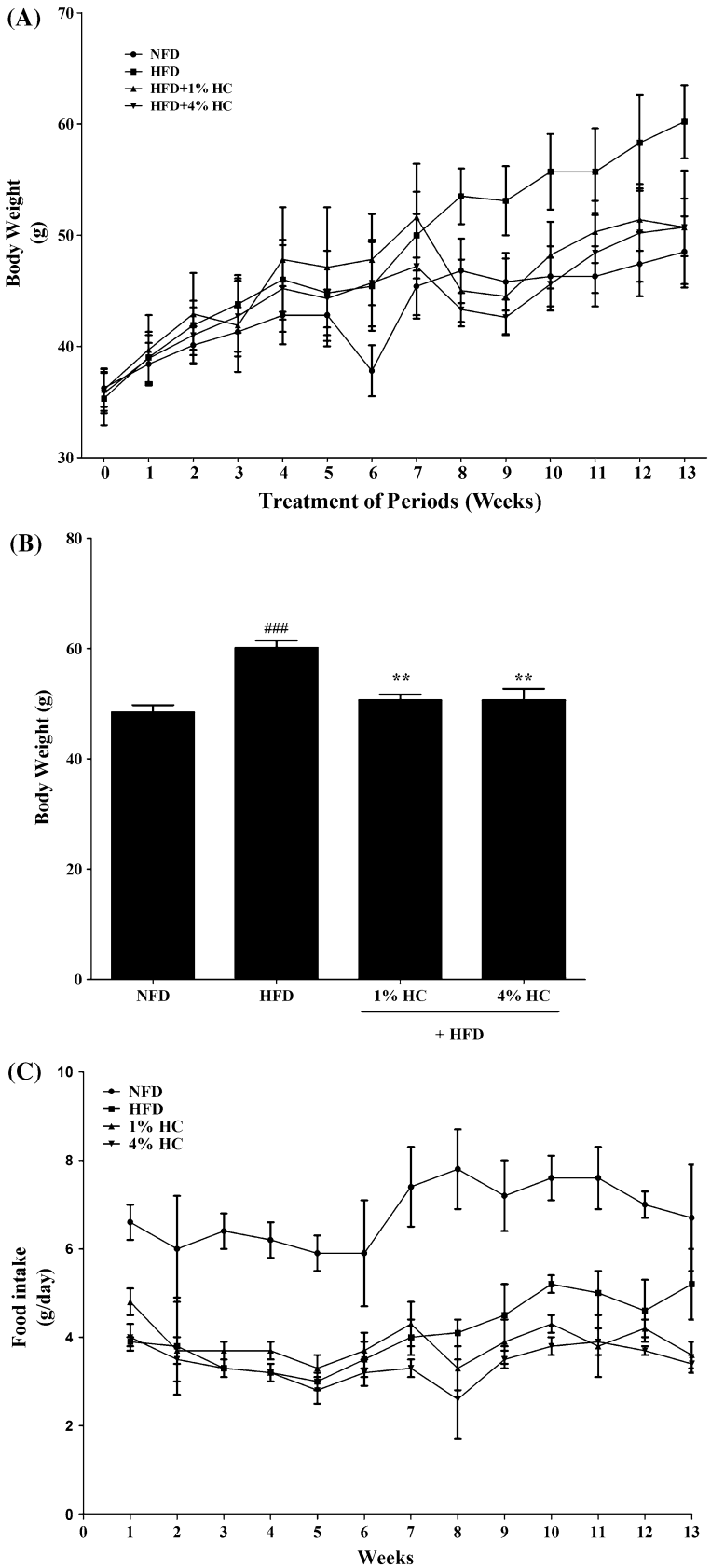

Fig. 2 Effect of HC on change of body weight and food intake in high-fat diet-fed mice. Mice were grouped ($n = 6$ per group) and fed as described. (**A**) Body weight gain of mice. (**B**) Body weight on 12 weeks. (**C**) Change of food intake

Table 1 Effects of HC on serum lipid contents in obese mice induced by high-fat diet

	Glucose (mg/dL)	AST (μg/mL)	ALT (μg/mL)	Triglycerol (μg/mL)	Total cholestrol (μg/mL)
NFD	115.0 ± 10.2[b]	239.4 ± 31.9[a]	107.6 ± 9.6[a]	9.8 ± 4.5[b]	66.4 ± 8.6[a]
HFD	158.0 ± 5.0[a]	58.4 ± 10.8[d]	50.2 ± 7.9[b]	26.6 ± 12.8[a]	86.4 ± 22.2[a]
HFD +1 % HC	95.8 ± 14.6[c]	155.2 ± 13.4[b]	66.0 ± 11.0[b]	27.4 ± 9.0[a]	83.6 ± 14.2[a]
HFD +4 % HC	89.4 ± 6.6[c]	114.0 ± 19.5[c]	45.3 ± 15.7[b]	29.7 ± 9.0[a]	70.0 ± 6.4[a]

Values are shown as the mean ± SEM ($n = 6$). Data are significantly different when compared to others group at $p < 0.05$. Groups were included *NFD* normal diet, *HFD* high-fat diet, HFD + 1 % HC: 1 % HC + HFD, HFD + 4 % HC: 4 % HC + HFD

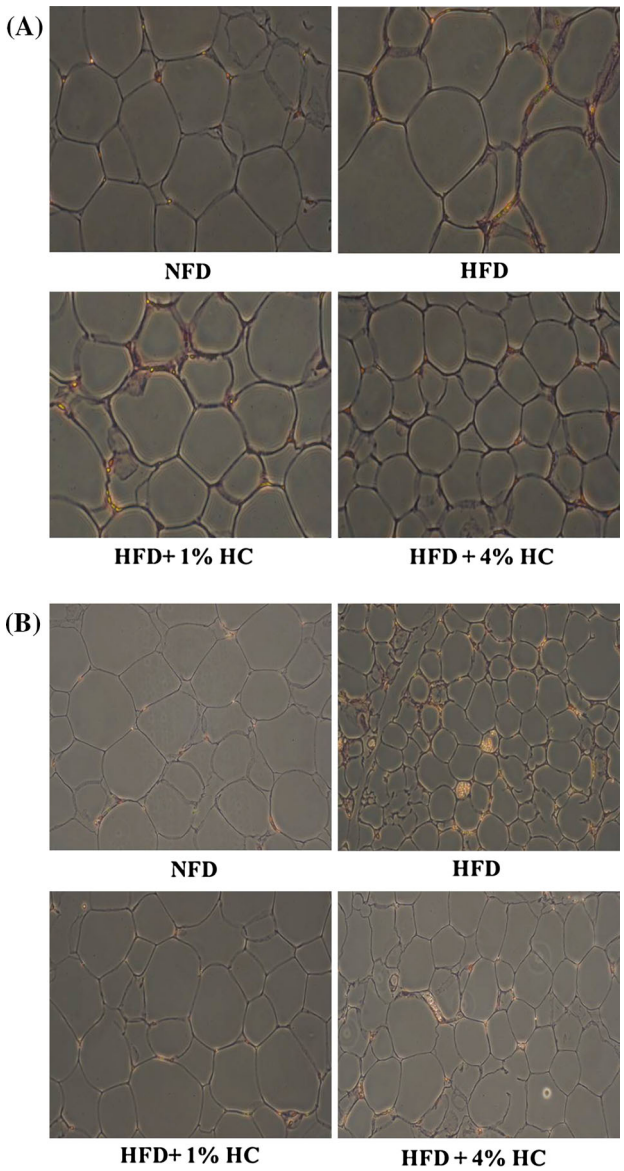

Fig. 3 Histological analyses of subcutaneous (**A**) abdominal (**B**) fat tissue in mice fed high-fat diet (HFD) with *Houttuynia cordata* (HC). H&E staining, magnification ×400 (**A**) or ×200 (**B**)

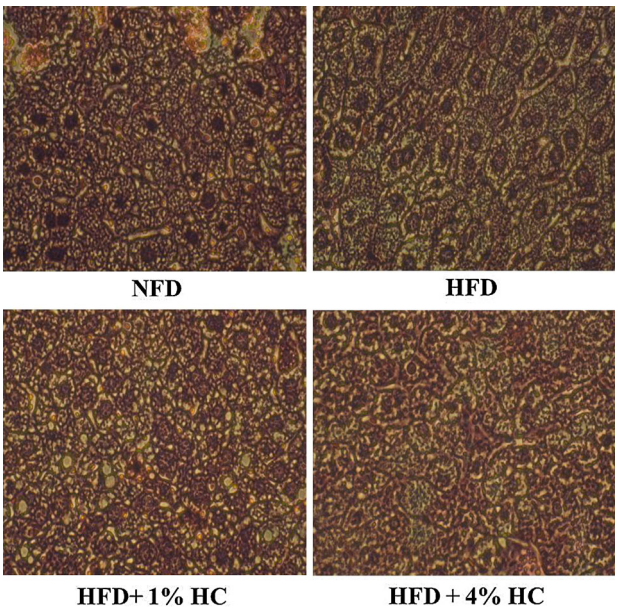

Fig. 4 Histological analyses in liver injury induced by high-fat diet in mice fed high-fat diet (HFD) with *Houttuynia cordata* (HC). H&E staining, magnification ×400

hepatic vacuolation by HC supplementation. Results from our study suggest that HC standardized extract could be a potential natural product to be associated with the improving liver damage in high-fat-induced mice.

Acknowledgments This work was supported by the Natl. Research Foundation of Korea (NRF) grant funded by the Korea government Ministry of Education, Science, Technology (No. 2014R1A2A2A01007627).

References

Aggarwal BB (2010) Targeting inflammation-induced obesity and metabolic diseases by curcumin and other nutraceuticals. Annu Rev Nutr 30:173–199

Aronne LJ, Isoldi KK (2007) Overweight and obesity: key components of cardiometabolic risk. Clin Cornerstone 8:29–37

Chen YY, Liu JF, Chen CM, Chao PY, Chang TJ (2003) A study of the antioxidative and antimutagenic effects of *Houttuynia cordata* Thunb using an oxidized frying oil-fed model. J Nutr Sci Vitaminol (Tokyo) 49:327–333

Cho AS, Jeon SM, Kim MJ, Yeo J, Seo KI, Choi MS, Lee MK (2010) Chlorogenic acid exhibits anti-obesity property and improves lipid metabolism in high-fat diet-induced-obese mice. Food Chem Toxicol 48:937–943

Chou SC, Su CR, Ku YC, Wu TS (2009) The constituents and their bioactivities of *Houttuynia cordata*. Chem Pharm Bull (Tokyo) 57:1227–1230

Han EH, Park JH, Kim JY, Jeong HG (2009) *Houttuynia cordata* water extract suppresses anaphylactic reaction and IgE-mediated allergic response by inhibiting multiple steps of FcepsilonRI signaling in mast cells. Food Chem Toxicol 47:1659–1666

Hayashi K, Kamiya M, Hayashi T (1995) Virucidal effects of the steam distillate from *Houttuynia cordata* and its components on HSV-1, influenza virus, and HIV. Planta Med 61:237–241

Hsu CL, Yen GC (2007) Effects of flavonoids and phenolic acids on the inhibition of adipogenesis in 3T3-L1 adipocytes. J Agric Food Chem 55:8404–8410

Hsu CL, Yen GC (2008) Phenolic compounds: evidence for inhibitory effects against obesity and their underlying molecular signaling mechanisms. Mol Nutr Food Res 52:53–61

Jang SY, Bae JS, Lee YH, Oh KY, Park KH, Bae YS (2011) Caffeic acid and quercitrin purified from *Houttuynia cordata* inhibit DNA topoisomerase I activity. Nat Prod Res 25:222–231

Ji L, Jiang P, Lu B, Sheng Y, Wang X, Wang Z (2013) Chlorogenic acid, a dietary polyphenol, protects acetaminophen-induced liver injury and its mechanism. J Nutr Biochem 24:1911–1919

Kim SK, Ryu SY, No J, Choi SU, Kim YS (2001) Cytotoxic alkaloids from *Houttuynia cordata*. Arch Pharm Res 24:518–521

Koriem KM, Soliman RE (2014) Chlorogenic and caftaric acids in liver toxicity and oxidative stress induced by methamphetamine. J Toxicol 2014:583494–583504

Kusirisin W, Srichairatanakool S, Lerttrakarnnon P, Lailerd N, Suttajit M, Jaikang C, Chaiyasut C (2009) Antioxidative activity, polyphenolic content and anti-glycation effect of some Thai medicinal plants traditionally used in diabetic patients. Med Chem 5:139–147

Lin MC, Hsu PC, Yin MC (2013) Protective effects of *Houttuynia cordata* aqueous extract in mice consuming a high saturated fat diet. Food Funct 4:322–327

Lu H, Wu X, Liang Y, Zhang J (2006) Variation in chemical composition and antibacterial activities of essential oils from two species of Houttuynia THUNB. Chem Pharm Bull (Tokyo) 54:936–940

Meng J, Leung KS, Jiang Z, Dong X, Zhao Z, Xu LJ (2005) Establishment of HPLC-DAD-MS fingerprint of fresh *Houttuynia cordata*. Chem Pharm Bull (Tokyo) 53:1604–1609

Meng J, Leung KS, Dong XP, Zhou YS, Jiang ZH, Zhao ZZ (2009) Simultaneous quantification of eight bioactive components of *Houttuynia cordata* and related *Saururaceae* medicinal plants by on-line high performance liquid chromatography-diode array detector-electrospray mass spectrometry. Fitoterapia 80:468–474

Mubarak A, Hodgson JM, Considine MJ, Croft KD, Matthews VB (2013) Supplementation of a high-fat diet with chlorogenic acid is associated with insulin resistance and hepatic lipid accumulation in mice. J Agric Food Chem 61:4371–4378

Negi AS, Kumar JK, Luqman S, Shanker K, Gupta MM, Khanuja SP (2008) Recent advances in plant hepatoprotectives: a chemical and biological profile of some important leads. Med Res Rev 28:746–772

Schaffer JE (2003) Lipotoxicity: when tissues overeat. Curr Opin Lipidol 14:281–287

Slawik MA, Vidal-Puig J (2006) Lipotoxicity, overnutrition and energy metabolism in aging. Ageing Res Rev 5:144–164

Unger RH, Orci L (2001) Diseases of liporegulation: new perspective on obesity and related disorders. Faseb J 15:312–321

Unger RH, Clark GO, Scherer PE, Orci L (2010) Lipid homeostasis, lipotoxicity and the metabolic syndrome. Biochim Biophys Acta 1801:209–214

Van Herpen NA, Schrauwen-Hinderling VB (2008) Lipid accumulation in non-adipose tissue and lipotoxicity. Physiol Behav 94:231–241

Xu CJ, Liang YZ, Chau FT (2005) Identification of essential components of *Houttuynia cordata* by gas chromatography/mass spectrometry and the integrated chemometric approach. Talanta 68:108–115

Xu X, Ye H, Wang W, Yu L, Chen G (2006) Determination of flavonoids in *Houttuynia cordata* Thunb. and *Saururus chinensis* (Lour.) Bail. by capillary electrophoresis with electrochemical detection. Talanta 68:759–764

Xu Y, Chen J, Yu X, Tao W, Jiang F, Yin Z, Liu C (2010) Protective effects of chlorogenic acid on acute hepatotoxicity induced by lipopolysaccharide in mice. Inflamm Res 59:871–877

Xu D, Hu L, Xia X, Song J, Li L, Song E, Song Y (2014) Tetrachlorobenzoquinone induces acute liver injury, up-regulates HO-1 and NQO1 expression in mice model: the protective role of chlorogenic acid. Environ Toxicol Pharmacol 37:1212–1220

Yang UJ, Ko SH, Shim SM (2014) Vitamin C from standardized water spinach extract on inhibition of cytotoxicity and oxidative stress induced by heavy metals in HepG2 cells. J Korean Soc Appl Biol Chem 57:161–166

Zhang LT, Chang CQ, Liu Y, Chen ZM (2011) Effect of chlorogenic acid on disordered glucose and lipid metabolism in db/db mice and its mechanism. Zhongguo Yi Xue Ke Xue Yuan Xue Bao 33:281–286