Preventive Effect of Fermented Chestnut Inner Shell Extract on Obesity-Induced Hepatic Steatosis

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ABSTRACT: This study aimed to investigate the effects of the gallic acid-enriched fermented chestnut inner shell extract (FCCE) by *Saccharomyces cerevisiae* on a high fat diet (HFD)-induced obesity and hepatic steatosis *in vivo* mouse model. Mice feeding FCCE exhibited reduced body weight gain compared to those in the HFD-fed group, and showed lower abdominal fat pad weight including epididymal, retroperitoneal, and mesenteric adipose tissue. Further, FCCE administration decreased adipocyte size by suppressing adipogenic factors such as peroxisome proliferator activated receptor γ and CCAAT/enhancer-binding protein α, and lipogenic factors such as sterol regulatory element-binding protein-1c, fatty acid synthase, and stearoyl CoA desaturase-1. Moreover, FCCE decreased levels of lipids in serum and liver as well as serum alanine aminotransferase and aspartate aminotransferase levels, markers of liver injury. Histological observations of the liver showed that FCCE significantly attenuated HFD-induced hepatic steatosis. The effect of FCCE on hepatic lipid regulatory factors may be partly associated with adenosine monophosphate-activated protein kinase activation. These results suggest that gallic acid-enriched FCCE has potential to be a promising functional food for prevention of obesity and obesity-related fatty liver disease.

Keywords: chestnut, fatty liver, fermentation, obesity

INTRODUCTION

Obesity, characterized by being severely overweight, is a major factor that increases the risk of serious metabolic diseases such as heart failure, hypertension, diabetes, and osteoarthritis. In general, obesity is established by expansion of adipose tissues owing to excessive caloric intake, which results in adipocyte hyperplasia and hypertrophy (Polyzos and Mantzoros, 2019). Adipose tissue develops and expands by adipocyte differentiation to store residual energy sources such as glucose and lipids; key factors in this process are CCAAT/enhancer-binding proteins β and δ (C/EBPβ and C/EBPδ, respectively), which induce transcription of C/EBPα and peroxisome proliferator activated receptor γ (PPARγ). PPARγ promotes lipogenesis in adipocytes via activating sterol regulatory element-binding protein 1c (SREBP-1c), which promotes transcription of lipogenic factors including fatty acid synthase (FAS) and stearoyl CoA desaturase 1 (SCD-1) (Guo et al., 2015). Excessive lipids can also be stored in the liver. Hepatic lipid accumulation exceeding 5% of liver weight, termed steatosis, is considered the earliest and most prominent diagnosis of non-alcoholic fatty liver disease (NAFLD), including range of steatohepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma (HCC) (Browning and Horton, 2004). High fat intake can cause hepatic steatosis by inducing an imbalance between triglyceride (TG) synthesis and fatty acid β-oxidation in the liver, which may lead to non-alcoholic steatohepatitis (NASH) accompanying inflammatory responses (Tessari et al., 2009). NAFLD ranges from severe fibrosis to HCC, and is irreversible, consequently leading to death (Younossi et al., 2015).

Chestnut (*Castanea crenata*) inner shell (CIS), an edible part of whole chestnut, is discarded as agricultural waste because of its astringent taste. However, CIS has benefits such as anti-wrinkling and whitening effects, and antioxidant activity (Jang et al., 2011; Jung et al., 2016). Moreover, CIS shows hepatoprotective activity against high fat diet (HFD)-induced fatty liver disease, which is considered to be mediated by the active components scopoletin

and scoparone, however, the content of these compounds in CIS are unknown (Noh et al., 2010). Previous reports have demonstrated that gallic acid and ellagic acid are the most abundant components in CIS extract (Son et al., 2005; Jeong et al., 2009).

Several studies have demonstrated that natural sources fermented by edible microorganisms, such as fermented green tea and turmeric, enhance their abilities for preventing metabolic disorders (Ho et al., 2012; Seo et al., 2015). A study investigating anti-allergic effects of CIS extracts fermented by *Lactobacillus bifermentans* has been reported (Choi et al., 2013). However, the effect of fermented CIS extracts on hepatic steatosis has not yet been reported. In this study, we established a procedure for developing gallic acid-enriched fermented CIS extract by *Saccharomyces cerevisiae* and suggested the possible preventive mechanism of CIS against HFD-induced fatty liver.

MATERIALS AND METHODS

Preparation of fermented CIS extracts

CIS (18 kg) was added to 50% ethanol (300 kg) and heated at 80°C for 4 h in a fermenter (MJS U3; Marubishi, Tokyo, Japan). The extract was filtered, concentrated, and sterilized for 30 min at 121°C. After cooling to 30°C, the extract was inoculated with edible microorganisms, including *S. cerevisiae*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *L. casei*, *Lactobacillus fermentans*, and *Bacillus subtilis*, at 5% (v/v), and then incubated for 24 h at 30~37°C. The fermented extracts were sterilized for 1 h at 100°C, then filtered, concentrated, and spray-dried. Non-fermented CIS extracts were used as the control during *in vitro* studies. The CIS extract fermented by *S. cerevisiae* (FCCE) was selected following the *in vitro* studies and used during the *in vivo* study.

Differentiation of 3T3-L1 cells and Oil red O staining

3T3-L1 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% calf serum. For adipocyte differentiation, cells were incubated for a further 48 h after cells reached full confluency. Culture medium was then replaced with differentiation medium containing 10 μg/mL insulin, 0.5 μM dexamethasone, 0.8 mM isobutylmethyl xanthine, and fermented CIS. After 4 days, the medium was replaced with growth medium and incubated for 4 days. The cells were fixed with 10% formalin at room temperature for 10 min and stained with Oil red O solution at room temperature for 1 h. The stained Oil red O in the cells were then dissolved by isopropanol and the absorbance was measured using a microplate reader at 490 nm.

Determination of antioxidant capacity and analysis of active compounds in FCCE

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and total phenolic compound contents were estimated according to the method described by Okawa et al. (2001) and Stratil et al. (2006), respectively. DPPH radical scavenging activity was calculated as percent reduction compared with the negative control. Total phenolic compound contents in extracts were calculated from the gallic acid standard curve, and the results were presented in gallic acid equivalent (GAE).

Gallic acid and ellagic acid (Sigma, St. Louis, MO, USA) in FCCE were analyzed by using high performance liquid chromatography (HPLC). The HPLC conditions for gallic acid analysis were as follows: the column used was a Capcell pak C18 (4.6×250 mm, 5 μm, 120Å; Shiseido, Tokyo, Japan); the column temperature was 40°C; the flow rate was 1.0 mL/min; the binary gradient elution system consisted of 0.1% acetic acid in water (A) and 0.1% acetic acid in acetonitrile (B) [0 min (5% B), 20 min (25% B), 21 min (100% B), 35 min (100% B), 36 min (5% B), and 50 min (5% B)]; the wavelength employed was 280 nm; and the injection volume was 5 μL. HPLC conditions for ellagic acid analysis were as follows: the column used was a Capcell pak C18 (4.6×250 mm, 5 μm, 120Å); the column temperature was 30°C; the flow rate was 0.5 mL/min; the binary gradient elution system consisted of 0.1% formic acid in water (A) and acetonitrile (B) [0 min (10% B), 5 min (10% B), 35 min (50% B), 40 min (10% B), and 45 min (10% B)]; the wavelength was 306 nm; and the injection volume was 5 μL.

Animal experiments

C57/BL6J mice were obtained from OrientBio (Seongnam, Korea). Animals were maintained in a controlled environment at 25±2°C under a 12 h dark/light cycle and were acclimated for one week. The animals were randomly divided into 3 groups (n=6): low fat diet (LFD, 10% kcal fat) group; HFD (60% kcal fat) group; and the HFD with FCCE (FCCE) group. Mice in the LFD and HFD groups were administered saline once a day orally. For mice in the FCCE group, FCCE was administered orally once a day at dose of 250 mg/kg body weight. Diet and water were supplemented *ad libitum* and food intake was determined daily. After 8 weeks, mice were sacrificed and serum was collected. The livers and abdominal adipose tissues were promptly removed and tissue samples were fixed in 10% formalin. The remaining tissues were stored at −80°C until use. The animal experiments were approved by the Institutional Animal Care and Use Committee of Korea University (KUIACUC-2016-150).

Serum biochemical assays and hepatic lipids analysis

The levels of serum alanine aminotransferase (ALT), as-

partate aminotransferase (AST), total TG, and cholesterol (TC) were determined using kits from Roche Diagnostics (Basel, Switzerland) according to the manufacturer's instructions. Liver (50 mg) was homogenized in lipid extract buffer (50 mM Tris-HCl, 150 mM NaCl, 10 mM ethylenediaminetetraacetic acid, and 0.1% Tween-20, pH 7.5) and the supernatant was used to quantify TG and TC.

Histological analysis

Paraffin embedded tissues were sectioned at 5 μm thickness, stained with hematoxylin and eosin (H&E), and Oil red O, and examined under a light microscope (Olympus, Tokyo, Japan). 4,4-Difluoro-1,3,5,7,8-pentamethyl-4-bora-3a,4a-diaza-S-indacene (BODIPY 493/503) fluorescein was used for neutral lipid staining under the fluorescence microscope.

Semi-quantitative real-time-polymerase chain reaction (semi-qRT-PCR) and qRT-PCR

Total RNA was isolated using the easy-BLUETM total RNA extraction kit (Intron Biotechnology, Inc., Seongnam, Korea) and reverse transcribed to cDNA using a cDNA synthesis kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA) as described in the manufacturer's protocol. For semi-qRT-PCR, PCR products were separated by electrophoresis on 1.8% agarose gel stained with ethidium bromide. PCR products were visualized under ultraviolet light and photographed using the gel documentation system. The intensities of the bands were quantified using ImageJ software [v1.32j; National Institute of Health (NIH), Bethesda, MD, USA]. Relative levels of expression of target genes were normalized to glyceraldehyde 3-phosphate dehydrogenase, and data were expressed as a ratio compared to control. For qRT-PCR, cDNA was mixed with SYBR master mix (Thermo Fisher Scientific, Inc.) and forward and reverse primers, and reaction were performed using ABI7500 system (Applied Biosystem, Foster, CA, USA). Expression was calculated using the $\triangle\triangle C_T$ method. The primers used are listed in Table 1.

Immunoblot analysis

Liver and adipose tissues were homogenized and lysed. Total protein concentrations were determined using Bradford assay. Equal amounts of protein were separated by electrophoresis and transferred onto polyvinylidene difluoride membranes. The membranes were incubated with primary antibodies overnight, followed by horseradish peroxidase-linked secondary antibodies for 1 h. Proteins were visualized using an enhanced chemiluminescence system (Thermo Fisher Scientific, Inc.) and band intensities were quantified with ImageJ software (v1.32j; NIH). Antibodies targeting FAS, acetyl-CoA carboxylase (ACC), pACC, AMP-activated protein kinase

Table 1. Primers used in polymerase chain reaction reactions

Gene		Sequences (5′→3′)
SREBP-1c	Forward	GATCGCAGTCTGAGGAGGAG
	Reverse	GATCGCCAAGCTTCTCTACG
FAS	Forward	ACTGGGTGACCTTCATGGAC
	Reverse	ACCACCAGAGACCGTTATGC
SCD-1	Forward	GTTCGTTAGCACCTTCTTGCG
	Reverse	TATCCATAGAGATGCGCGGC
PPARγ	Forward	GGGGATGTCTCACAATGCCA
	Reverse	GATGGCCACCTCTTTGCTCT
C/EBPα	Forward	GCAAAGCCAAGAAGTCGGTG
	Reverse	TCACTGGTCAACTCCAGCAC
C/EBPβ	Forward	GAAGACGGTGGACAAGCTGA
	Reverse	GCTTGAACAAGTTCCGCAGG
GAPDH	Forward	GTCAAGGCTGAGAACGGGAA
	Reverse	AAATGAGCCCCAGCCTTCTC

SREBP-1c, sterol regulatory element-binding protein-1c; FAS, fatty acid synthase; SCD-1, stearoyl CoA desaturase-1; PPARγ, peroxisome proliferator activated receptor γ; C/EBP, CCAAT/enhancer-binding protein; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

(AMPK), and pAMPK (Thr172) were purchased from Cell Signaling (Danvers, MA, USA). Antibodies targeting SREBP-1c, PPARα, SCD-1, and β-actin were purchased from Santa Cruz Biotechnologies (Dallas, TX, USA).

Statistical analysis

Differences between samples were analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range post-hoc test using the Statistical Analysis System (SAS) program (SAS 8.2, SAS Institute Inc., Cary, NC, USA).

RESULTS

Screening the effects of fermented CIS extracts on adipocyte differentiation and antioxidant activity

To determine the inhibitory effects of fermented CIS extracts on 3T3-L1 differentiation, the levels of lipid in mature 3T3-L1 cells were analyzed by Oil red O staining. As shown in Fig. 1A, non-fermented CIS extract only reduced lipid accumulation in differentiated 3T3-L1 cells by 9%. However, CIS extracts fermented by *S. cerevisiae*, *L. acidophilus*, *L. plantarum*, *L. casei*, *L. fermentans*, and *B. subtilis* reduced lipid accumulation by 42%, 29%, 33%, 30%, 30%, and 34%, respectively. CIS extract fermented by *S. cerevisiae* was the most effective for inhibiting adipocyte differentiation. Non-fermented extracts showed 37% radical scavenging activity, however, CIS extracts fermented by *S. cerevisiae* and *L. plantarum* significantly increased radical scavenging activity by 49.4% and 44.3%, respectively (Fig. 1B). The total phenolic content in non-fermented extracts was 4.2 μg GAE/100 μg (Fig. 1C);
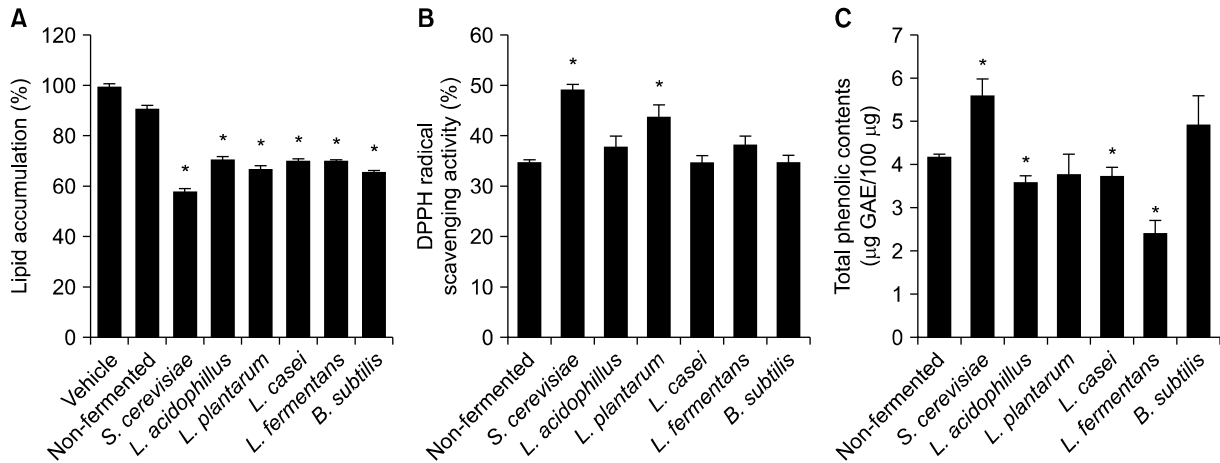
Fig. 1. Adipocyte differentiation inhibitory activity and anti-oxidant activity of fermented chestnut inner shell extracts. (A) Lipid contents of differentiated 3T3-L1 adipocyte were determined by Oil red O staining. Vehicle indicates the distilled water-treated group as a control. Test samples were treated at a concentration of 0.5 mg/mL. Data are expressed as means±SD. (B) 1,1-Di-phenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of the test sample as tested at a concentration of 0.5 mg/mL. (C) Total phenolic contents in test samples were quantified from standard curve using gallic acid. Data are expressed as gallic acid equivalent (GAE). Significant difference versus the vehicle (A) or non-fermented extract (B and C) at $P<0.05$.

Table 2. High performance liquid chromatography analysis of gallic acid and ellagic acid contents in non-fermented and fermented chestnut inner shell extracts (unit: mg/g)

	Non-fermented	FCCE
Gallic acid	2.19±0.05	6.68±0.26*
Ellagic acid	9.23±0.16	4.27±0.23*

Data are expressed as mean±SD.
Significant difference between non-fermented extract and FCCE (*$P<0.05$).
FCCE, fermented chestnut inner shell extract by *Saccharomyces cerevisiae*.

Table 3. Effect of fermented chestnut inner shell extract (FCCE) on weight gain, food intake, calorie intake, food efficiency ratio (FER), and serum lipid level during the experimental period

Parameters	Experimental groups		
	LFD	HFD	FCCE
Weight gain (g)	8.3±1.9*	24.3±3.2	17.7±2.2*
Food intake (g/d)	2.8±0.4*	2.6±0.4	2.6±0.4
Caloric intake (kcal/d)	5.5±1.0*	11.1±1.9	11.0±1.7
FER (%)	5.2±1.2*	16.4±2.4	12.0±1.7*
Serum TG (mg/dL)	26.6±9.2*	72.0±8.5	39.1±5.0*
Serum TC (mg/dL)	114.5±17.6*	203.8±11.2	163.0±17.1*

Data are expressed as mean±SD.
Significant differences versus HFD group (*$P<0.05$).
FER=(body weight gain/ total food intake)×100.
LFD, low fat diet fed group with oral administration of saline; HFD, high fat diet fed group with oral administration of saline; FCCE, high fat diet fed group with oral administration of FCCE 250 mg/kg.

total phenolic content was increased to 5.6 μg GAE/100 μg in extracts fermented by *S. cerevisiae* but reduced to 3.6, 3.8, and 2.5 μg GAE/100 μg in extracts fermented by *L. acidophilus*, *L. casei*, and *L. fermentans*, respectively. Thus, we selected fermented CIS extract using *S. cerevisiae* (FCCE) for further study since it showed the strongest inhibitory effect on adipocyte differentiation and antioxidant capacity. We analyzed the quantity of gallic acid and ellagic acid as main phenolic acids in non-fermented CIS extract and FCCE by HPLC. As shown in Table 2, fermentation increased gallic acid in samples from 2.19 mg/g to 6.68 mg/g, and decreased ellagic acid from 9.23 mg/g to 4.27 mg/g.

Effect of FCCE on HFD-induced obesity

Body weight gain during experimental periods was significantly lower in the FCCE group (17.7 g) than that of HFD-fed group (24.3 g) (Table 3). However, there were no significant differences in the daily food intake and calorie intake between the HFD and FCCE groups. Furthermore, FER, serum TG levels, and serum TC levels were lower in the FCCE group compared with the HFD group.

Effect of FCCE on adipose tissue in HFD-induced obese mice

As shown in Fig. 2A, the change in body weight at week 8 was significantly lower for the FCCE group compared with the HFD group. The weights of epididymal, retroperitoneal, and mesenteric adipose tissues (EAT, RAT, and MAT, respectively) were significantly lower for mice in the FCCE group compared with the HFD group (Fig. 2B). Further, the areas and diameters of epididymal adipocytes were higher for mice in the HFD group compared with those in the LFD group. However, administration of FCCE significantly reduced HFD-induced adipocyte hypertrophy (Fig. 2C and 2D). To investigate whether the reduction in abdominal adipose tissues in mice receiving the HFD and treated with FCCE is related to regulation of adipogenic and lipogenic factors, qRT-PCR was performed. Expression of adipogenic factors (C/EBPα and
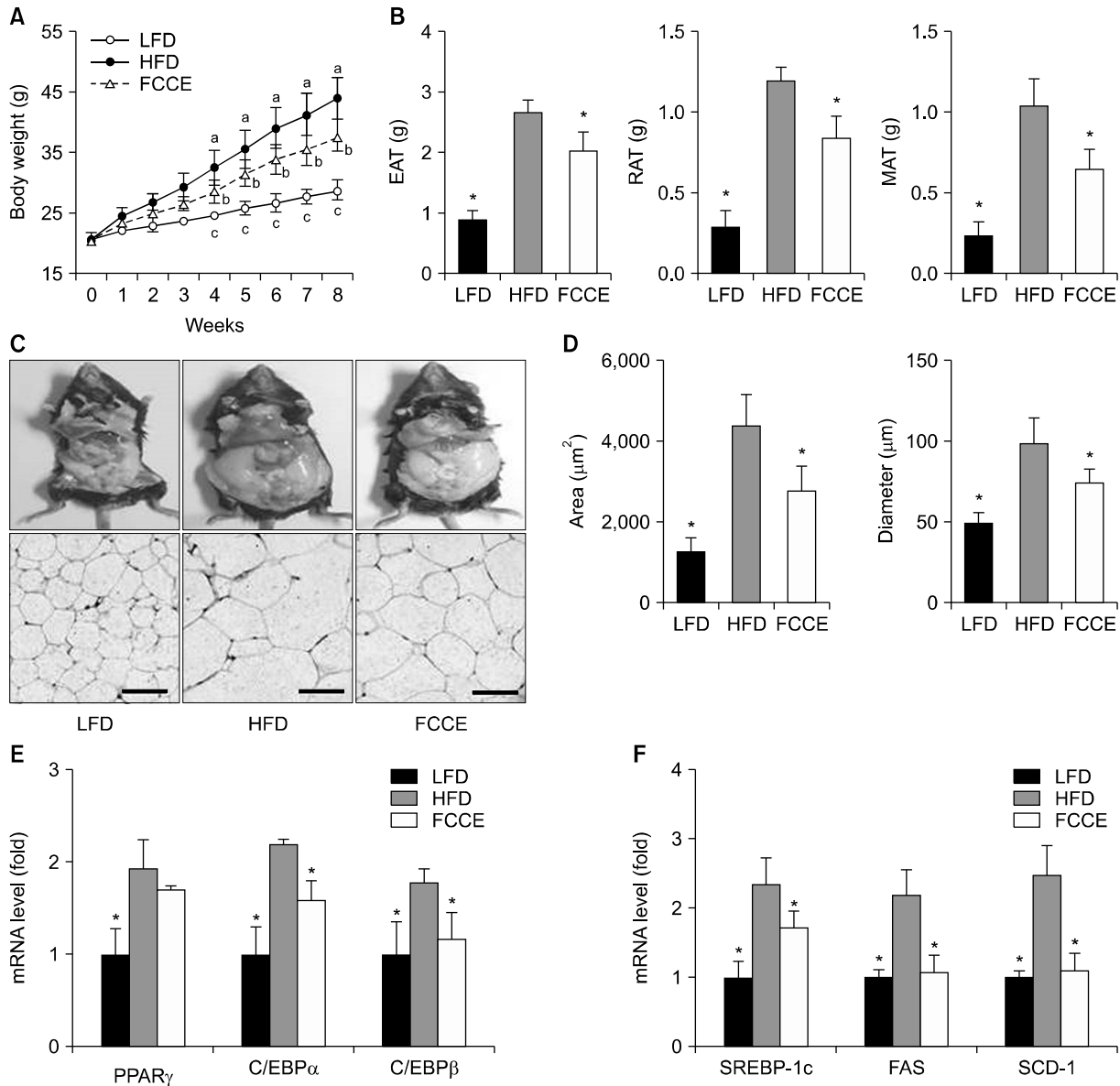
Fig. 2. Anti-obesity effect of fermented chestnut inner shell extract (FCCE) in high fat diet (HFD)-induced obese mice. (A) Body weight change during the experimental periods. EAT, eipididymal adipose tissue; RAT, retropheritoneal adipose tissue; MAT, mesenteric adipose tissue. (B) Abdominal adipose tissue weights. (C) Hematoxylin and eosin staining of epididymal adipose tissue section (scale bar=100 μm). (D) The area and diameter of epididymal adipocyte (E and F). Expression of adipogenic genes [peroxisome proliferator activated receptor (PPAR) α, CCAAT/enhancer-binding protein (C/EBP) α, and C/EBPβ] and lipogenic genes [sterol regulatory element-binding protein-1c (SREBP-1c), fatty acid synthase (FAS), and stearoyl CoA desaturase-1 (SCD-1)] in epididymal adipose tissue. Genes level were analyzed by quantitative real-time-polymerase chain reaction. Data are expressed as mean±SD. Significant differences versus HFD group (*$P<0.05$). LFD, low fat diet fed group; HFD, HFD fed group; FCCE, high fat diet fed group with oral administration of FCCE 250 mg/kg.

C/EBPβ) and lipogenic factors (SREBP-1c, FAS, and SCD-1) were significantly increased in the HFD-fed group compared with the LFD-fed group (Fig. 2E and 2F). However, FCCE administration significantly down-regulated expression of these factors compared with the mice just receiving the HFD.

Effect of FCCE on hepatic steatosis induced by HFD

HFD-induced hepatic lipid accumulation was clearly observed by H&E, Oil red O, and BODIPY staining of liver sections (Fig. 3A). FCCE substantially reduced lipid ac-

cumulation in the livers of mice receiving the HFD. Hepatic TG and TC were also increased in the livers of HFD-fed mice, however these factors were significantly decreased in mice in the FCCE group. Moreover, levels of serum AST and ALT (markers for liver injury) were significantly increased in HFD-fed group, but significantly reduced in the FCCE group (Fig. 3B). Moreover, using the intraperitoneal glucose tolerance test, insulin resistance was shown to be induced by the HFD, and FCCE administration significantly improved the HFD-induced insulin resistance (Fig. 3C).
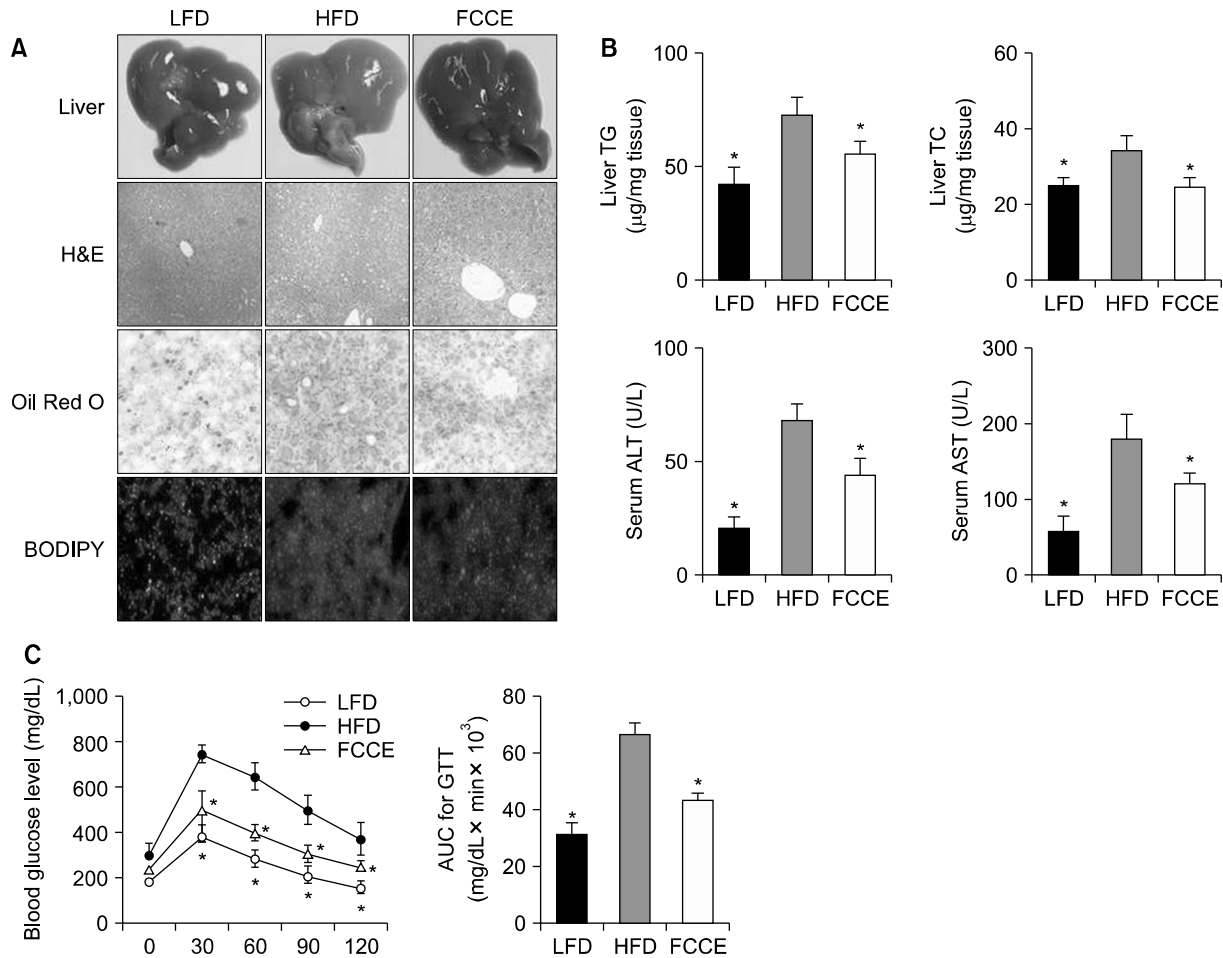
Fig. 3. Fermented chestnut inner shell extract (FCCE) attenuated hepatic lipid accumulation and insulin resistance in high fat diet (HFD)-induced obese mice. (A) Macroscopic liver images, hematoxylin and eosin, Oil red O, and BODIPY staining of liver section. In BODIPY staining, green color is neutral lipid-specific fluorescence probe (BODIPY) and blue color is nuclei-specific fluorescence probe (DAPI) (magnification ×100). (B) Hepatic triglyceride (TG), total cholesterol (TC), serum alanine aminotransferase (ALT), and aspartate aminotransferase (AST) levels. (C) Area under the curve (AUC) for oral glucose tolerance test (GTT). Data are expressed as mean±SD. *$P<0.05$ versus HFD group. LFD, low fat diet fed group; HFD, high fat diet fed group; FCCE, high fat diet fed group with oral administration of FCCE 250 mg/kg.

The effect of FCCE on hepatic lipogenesis was measured by examining lipogenic genes and protein expression. As shown in Fig. 4A and 4B, the HFD up-regulated gene and protein expression of SREBP-1c, FAS, and SCD-1, but these were downregulated by administration of FCCE. Further, FCCE stimulated phosphorylation of ACC and AMPK, and upregulated levels of PPARγ co-activators-1α protein in the liver.

DISCUSSION

Several studies have demonstrated that fermentation of natural sources with *Lactobacillus* or yeast enhances the anti-obesity effects by changing the contents of their active components (Wang et al., 2015; Zhang et al., 2017). In the present study, we established gallic acid-enhanced FCCE by fermenting with *S. cerevisiae* and examined its ability to prevent obesity-induced hepatic steatosis. It is well known that gallic acid and ellagic acid are abundant major phenolic acids in CIS extract, and have the preventive effects on HFD-induced obesity and hepatic steatosis through their diverse activities on lipid metabolism regulatory factors (Hsu and Yen, 2007; Panchal et al., 2013). In the present study we showed that ellagic acid is more abundant than gallic acid in non-fermented CIS extract. However, fermentation decreased ellagic acid 2-fold and increased gallic acid 3-fold (Table 2). Interestingly, after fermentation by *S. cerevisiae*, the antioxidant capacity of CIS and the inhibitory effect of CIS on 3T3-L1 preadipocyte differentiation was enhanced despite the decreased ellagic acid content (Fig. 1). Several studies have shown that total phenolic compounds are significantly increased after fermentation using *Lactobacillus* strains (Kwaw et al., 2018; Jung et al., 2019). Based on these results, the enhanced gallic acid content could be considered, at least in part, as the major active component of FCCE for preventing obesity.
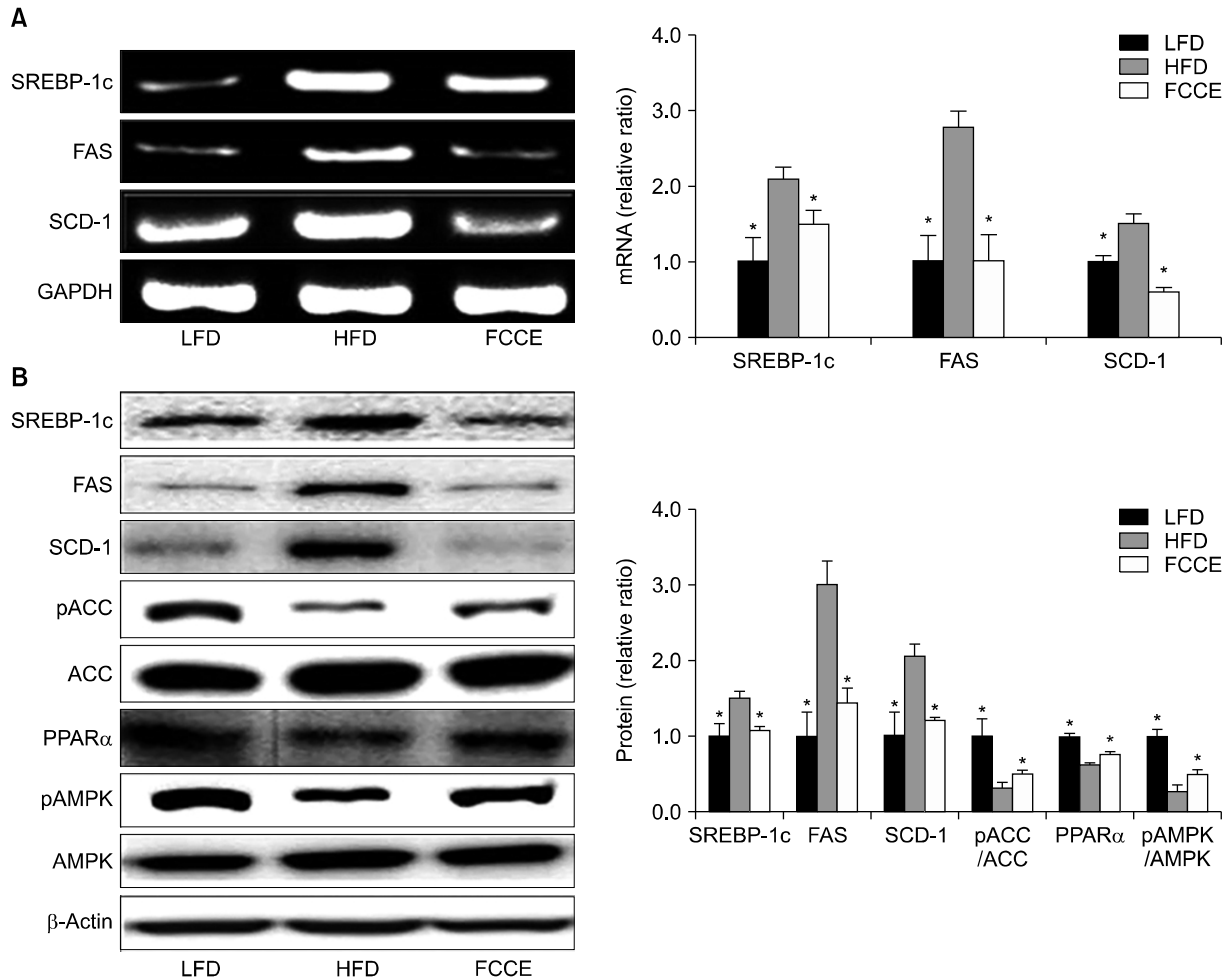
Fig. 4. Effect of fermented chestnut inner shell extract (FCCE) on lipid regulatory factors in liver of high fat diet (HFD)-fed obese mice. (A) Lipogenic genes were determined by real-time-polymerase chain reaction. (B) Lipid metabolism regulators were detected by western blot. Data are expressed as mean±SD. *P<0.05 versus HFD group. LFD, low fat diet fed group; HFD, high fat diet fed group; FCCE, high fat diet fed group with oral administration of FCCE 250 mg/kg. SREBP-1c, sterol regulatory element-binding protein-1c; FAS, fatty acid synthase; SCD-1, stearoyl CoA desaturase-1; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; ACC, acetyl-CoA carboxylase; PPAR, peroxisome proliferator activated receptor; AMPK, adenosine monophosphate-activated protein kinase.

Obesity is closely related to excessive fat intake exceeding metabolic needs. Hypertrophy of abdominal adipose tissue following excessive fat intake is the most representative feature of obesity (Mistry et al., 2015). In the present study, symptoms of obesity, including an increase of body weight, hyperlipidemia, and adipocyte hypertrophy were markedly attenuated in a HFD-fed animal model by oral FCCE administration. Over the experimental period, body weight gain was significantly decreased by FCCE administration despite mice consuming the same amount of food daily same caloric values as the HFD group, indicating that FCCE effectively reduces body weight gain without anorectic effects. Moreover, FCCE markedly decreased serum TG and TC levels that were increased by the HFD. A previous report demonstrated that anti-obesity effects of gallic acid results from its ability to inhibit pancreatic lipase (Oi et al., 2012). This indicates that the ability of FCCE to protect against HFD-induced obesity may be related to the enrichment of gallic acid.

In adipocytes, PPARγ and C/EBPα are critical transcription factors for adipocyte differentiation (Lefterova and Lazar, 2009; Lefterova et al., 2008). In the late stage of adipogenesis, C/EBPα promotes lipogenesis by activating PPARγ (Semenkovich, 1997). Activated PPARγ promotes expression of lipogenic proteins, such as SREBP-1c, FAS, and SCD-1, which mediate *de novo* fatty acid synthesis and lipid accumulation in adipose tissue (Evans et al., 2004; Tontonoz and Spiegelman, 2008). In the present study, FCCE attenuated adipocyte hypertrophy through down-regulating adipogenic factors, consequently decreasing adipocyte size. Gallic acid shows anti-obesity effect through inhibiting 3T3-L1 preadipocyte proliferation via inducing apoptosis (Hsu et al., 2007). Another study has demonstrated that gallic acid reduces weight gain and improves glucose homeostasis through activating AMPK (Doan et al., 2015). Therefore, the anti-adipogenic activity of FCCE may be tightly related to the anti-obesity ef-

fect of gallic acid.

NAFLD is characterized by excessive lipid accumulation in liver, which aggravates metabolic liver function impairing hepatic glucose and lipid metabolism (Yoon et al., 2014). Modulating lipid metabolism is therefore critical for preventing progression of NAFLD. In line with a previous report that demonstrated gallic acid strongly inhibits lipid accumulation in the liver (Hsu and Yen, 2007), the inhibitory effect of FCCE against HFD-induced hepatic steatosis may be closely related to the enriched gallic acid content following fermentation. Lipid metabolism homeostasis is regulated by the balance between hepatic *de novo* lipogenesis and fatty acid oxidation. SREBP-1c and PPARα are the key regulators in the lipid synthesis and fatty acid oxidation pathways, respectively (Musso et al., 2009). SREBP-1c is a transcription factor that stimulates hepatic lipid synthesis through up-regulating lipogenic enzymes such as FAS, SCD, and ACC (Yang et al., 2014). SREBP-1c is constitutively elevated in obese individuals, and this induces TG accumulation in the liver (Shimomura et al., 1999). Phosphorylated AMPK phosphorylates ACC, which inactivates its enzymatic activity to inhibit lipogenesis and stimulate fatty acid β-oxidation (Jung et al., 2011). PPARα stimulates mitochondrial fatty acid β-oxidation by upregulating carnitine palmitoyltransferase I and inhibits lipid synthesis through activating AMPK, subsequently preventing excessive lipid accumulation in the liver (Giby and Ajith, 2014). In the present study, FCCE inhibited lipid accumulation in the liver through down-regulating SREBP-1c and its downstream lipogenic factors, including FAS and SCD. A previous report has demonstrated that gallic acid attenuates HFD-induced hepatic steatosis through enhancing glucose and lipid metabolic pathways (Chao et al., 2014). Gallic acid attenuates HFD-induced impairment of lipid metabolism through AMPK-dependent pathway (Doan et al., 2015). These studies partly support that the attenuating effect of FCCE on hepatic lipogenesis may be linked to the enhanced AMPK activation.

Taken together, the present study demonstrates that FCCE prevents HFD-induced obesity by inhibiting adipogenesis and hepatic steatosis via activating AMPK. These results may partly result from the diverse effect of gallic acid on metabolic disease. We therefore suggest that gallic acid-enriched FCCE could be a promising functional food for preventing HFD-induced fatty liver disease. However, we were unable to elucidate the mechanism of action of FCCE because its preventive activity against hepatic steatosis is not restricted by the effect of gallic acid. A broader range of compounds in FCCE, including other phenolic acids, need to be analyzed to identify all the specific active components in FCCE.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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