J East Asian Soc Diet Life
29(6): 511 ~518 (2019)
http://dx.doi.org/10.17495/easdl.2019.12.29.6.511

Amelioratory Effects of Acorn *Mook* Ethanol Extract on ROS Production and Lipid Accumulation in Differentiated 3T3-L1 Cells

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ABSTRACT

Obesity is a cause of various metabolic diseases, so prevention and treatment are important. Recently, there has been an increasing interest in natural foods that promote fat breakdown or inhibit fat accumulation. The objective of this study was to investigate the amelioratory effect of acorn and acorn *mook* ethanol extract on reactive oxygen species (ROS) production and lipid accumulation in differentiated 3T3-L1 cells. First, antioxidant activity and the expression of C/EBP-β, C/EBP-α, PPAR-γ, and aP2 level by western blotting in 3T3-L1 cells of acorn ethanol extract were examined. Total polyphenol and total flavonoid contents of acorn ethanol extract were 476.44±14.70 mg TAE/g and 102.91±13.14 mg QE/g, respectively. AEtOH suppressed C/EBP-β, C/EBP-α, PPAR-γ expression, an adipogenesis related transcription factor, and inhibited aP2 expression in 3T3L-1 cells. Based on the first results, we made acorn *mook* and treated 3T3-L1 cells with acorn *mook* ethanol extract to investigate its inhibitory effect on ROS production and lipid accumulation. Acorn *mook* ethanol extract significantly inhibited ROS production and lipid accumulation in 3T3-L1 cells. These results suggest that acorn *mook* might be an effective potential agent to ameliorate obesity.

Key words: acorn *mook* ethanol extract, 3T3-L1 cells, ROS production, lipid accumulation

INTRODUCTION

Obesity has become a global health problem not only increases medical costs but also causes a reduction in the economically active population (Ng M *et al* 2015). In 2016, obesity prevalence of male in Korea was 42.3%, respectively (KCDC 2016). Obesity poses a serious threat to our health because it causes several diseases like metabolic syndrome, cardiovascular diseases, diabetes, hypertension, stroke, etc (Chukir T *et al* 2018; Reho JJ *et al* 2017; Murphy N *et al* 2018). Adipocytes are specialized cells that accumulate fat in adipose tissue. They are instrumental in energy homeostasis in the body (White UA & Tchoukalova YD 2014). Obesity develops when fat is excessively accumulated in adipose tissue. Differentiation of adipocytes precede obesity. For this reason, differentiation of 3T3-L1 cells (preadipocytes derived from mouse embryos) has been used to study molecular mechanisms of anti-obesity activity *in vitro* (Romacho T *et al* 2014). Adipogenesis is also controlled by adipogenic transcription factors such as peroxisome proliferator-activated receptor γ (PPAR γ) and CCAAT/enhancer-binding protein α (C/EBPα) (Romacho T *et al* 2014). These transcription factors can mediate the regulation of several genes necessary for the adipocyte phenotype and they cause adipogenesis (Rizzatti V *et al* 2013). Methods of managing obesity include diet therapy, medication, and surgery. However, surgery and pharmacotherapy have side effects, and in recent years there is an increasing interest in natural products that can safely and effectively control obesity (Chukir T *et al* 2018). Acorn (*Quercus acutissima* Carr.) is mostly found in parts of Europe and Asia. In Korea, acorns have been traditionally consumed in the form of *mook* using acorn starch as emergency food (Kim JO & Lee MJ 1976). Many studies have reported that whole acorn contains various polyphenols, including gallic acid, digallic acid, gallotannin, and tannin (Sung IS *et al* 1997; Moon HR *et al* 2013). However, there is no study on the possibility of acorn *mook* as an antiobesity food by inhibiting fat accumulation in fat cells. Therefore, in this study, we researched the effect acron *mook* on the production reactive oxygen species (ROS) and lipid accumulation in differentiated 3T3 L1 cells.

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MATERIALS AND METHODS

1. Preparation of Samples

Dried whole acorns without outer peel were purchased from Cholokmayl Co. (Seoul, Korea). These acorns were lyophilized acorn and ground into powder. Acorn powder was then extracted with 70% aqueous ethanol (1:10, w/v) by vortexing for two hours at room temperature followed by centrifugation at 10,620 g for 10 min. After filtering ethanol acorn extract (AEtOH) through a 0.22 μm PIFE filter unit, the extract was then concentrated using a rotary vacuum evaporator (N-1110, EYELA, Tokyo, Japan). Samples were then freeze-dried by storing in a deep freezer (WUF-500; Daihan Scientific Co., Gangwon, Korea). They were stored until anti-oxidation analysis and cell culture.

2. Antioxidant Activities of AEtOH

Total polyphenol content of acorn ethanol extract (AEtOH) was analyzed with the method described by Gutfinger (Gutifinger T & Letan A 1974) with slight modifications. Briefly, 50 μL of 2 N Folin-Ciocalteu reagent was added to 500 μL of extract. The mixture was left at room temperature for 3 minutes. Then 500 μL of 20% Na_2CO_3 solution was added to the mixture followed by incubation at 25℃ for 1 hour. Thereafter, 100 μL of this mixture was collected for ELISA. Absorbance was measured at wavelength of 725 nm on Tecan Infinite M200Pro plate reader (Green Mate Bio, Seoul, Korea). The absorbance value was used to calculate total polyphenol content by creating a standard curve with tannic acid (TAE). Total polyphenol content is expressed as mg TAE/g. Total flavonoid contents of acorn ethanol extract (AEtOH) was measured by Moreno MI *et al* (Moreno MI *et al* 2000). Briefly, sample solution (0.5 mL) was mixed with 1.5 mL ethanol (95%, v/v), 0.1 mL of aluminum chloride (10%, w/v), 0.1 mL of potassium acetate (1 M), and distilled water. After incubation at room temperature for 30 min, absorbance was determined at 415 nm. A calibration curve was prepared using rutin. Results are expressed as mg of rutin equivalents (mg RE/g). Antioxidant scavenging activity was measured using 1, 1-diphenyl,2-picrylhydrazyl free radical (DPPH). Various concentration of test solution in 0.1mL was added to 0.9 mL of 0.1 mM of DPPH solution in ethanol. Only ethanol (0.1 mL) was used as experimental control. After 30 min of incubation at room temperature, absorbance was measured at wavelength of 517 nm. The scavenging activity of the sample corresponded to the intensity of quenching DPPH. Percent scavenging activity was computed using the following equation:

$$\text{Scavenging activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

ABTS radical cations were produced by reacting ABTS (7 mM) and potassium persulfate (2.45 mM) at room temperature in the dark for 16 hours. The solution obtained was further diluted with PBS to give an absorbance of 1.000. Test sample (50 μL) at different concentrations was then added to ABTS working solution (950 μL) to give a final volume of 1 mL. Absorbance was recorded immediately at 734 nm (Auddy B *et al* 2003).

For ORAC assay, an automated plate reader (KC4, Bio Tek, USA) and 96-well plates were used (Prior RL *et al* 2003). Assays were conducted in phosphate buffer (pH 7.4) at 37℃. Peroxyl radical was generated using 2,2'-azobis (2-amidino-propane) dihydrochloride prepared fresh for each run. Fluorescein was used as substrate. Fluorescence conditions were as follows: excitation, 485 nm; emission, 520 nm. The standard curve was linear between 0 and 50 μM of Trolox. Results are expressed as μM TE/g fresh mass.

3. Cell Culture and Differentiation

3T3-L1 preadipocytes were purchased from the Korean Cell Line Bank (KCLB, Seoul, Korea). They were cultured in Dulbecco's modified Eagle's medium (DMEM, Hyclone, Thermo scientific, Waltham, MA, USA) supplemented with 10% (v/v) fetal bovine serum (FBS, Hyclone Laboratories INC, Gibco, NY, USA) and 1% (w/v) penicillin-streptomycin at 37℃ in a humidified 5% CO_2 atmosphere incubator (Thermo scientific, Waltham, MA, USA). Two days later, confluent cells were placed in differentiation medium containing 1 μg/mL insulin (I9278, Sigma, St. Louis, MO, USA), 0.5 mM IBMX [3-isobutyl-1-methyl xanthine (I7018, Sigma), and 1 μM dexamethasone (D4902, Sigma). The medium was subsequently replaced with fresh DMEM medium with 1 μg/mL insulin (I9278, Sigma). It was replaced

again with fresh DMEM medium containing 10% (w/v) FBS.

4. Cell Cytotoxity

3T3-L1 cells were seeded into 96-well plates at a density of 1×10^4 cells/well and cultured at 37℃ under 5% CO_2 for 24 h. Cells were then treated with different concentrations (0, 100, 300, and 500 μg/mL) of AEtOH extract. Following incubation for 24 h, cells were mixed with MTT solution (120 μL/well) and cultured for 4 h. Cell viability was determined based on difference in absorbance at wavelength of 540 nm (BN 02514, Molecular Device, Toronto, Canada).

5. Western Blotting Analysis

AEtOH treated 3T3-L1 cells were washed twice with ice-cold PBS and harvested with 0.25% (w/v) trypsin/EDTA on day 8. To prepare whole-cell extracts, cells were lysed in 50 μL of cell lysis buffer for 30 min on ice. Protein concentration of cell lysate was determined with Bradford method (Bradford MM 1976). Equal amounts of protein were subjected to 12% (w/v) SDS-polyacrylamide gel electrophoresis (Mini Protein Tetra Cell; Bio-Rad) and transferred onto nitrocellulose membrane (Mini Trans-Blot Transfer Cell; Bio-Rad) which was washed once with washing buffer (TBST, 0.1% (v/v) Tween 20). After blocking with a blocking buffer (PBS, 5% (w/v) skim milk, 0.05% (v/v) Tween 20) for 1 h, membranes were incubated with primary antibodies for 8 h at 4℃. Mouse polyclonal antibodies (C/ EBP-β, PPAR-γ, C/EBP-α, aP2, Santa Cruz) were diluted 1:1,000. Mouse polyclonal anti-β-actin (sc-47778, Santa Cruz) was diluted 1:2,000 in blocking buffer. After incubation, membranes were washed twice with washing buffer and subsequently incubated with goat anti-rabbit IgG HPR-conjugated secondary antibody (sc-2004; Santa Cruz, diluted 1:5,000 in blocking buffer) at room temperature for 1 h. After washing membranes twice with washing buffer, protein bands were detected using enhanced chemiluminescence (ECL, Amersham Pharmacia Biotech; Buckinghamshire, UK) and exposed to X-ray film (Eastman Kodak; Rochester, NY, USA). Band intensities were quantified with Scion-Image for Windows (National Institutes of Health, Bethesda, MD, USA).

6. Acorn *Mook* Processing and Preparation of Acorn *Mook* Ethanol Extracts

Ingredients of acorn *mook* were: 40 g of dried whole acorn (*Quercus acutissima* Carr.) powder, 60 g of mung bean starch, 3 g of salt, and 900 mL of water. Acorn *mook* was processed as shown in Fig. 1 and 2. All ingredients were mixed together and soaked for 3 hrs at room temperature (stage 1). Soaked ingredients were boiled at 100℃ for 8 min and heated at 80℃ for 20 min (stage 2). Finally, acorn *mook* product was poured into a 15 cm × 15 cm × 4.5 cm square bottle, left at room temperature for 1 hr, and then left at 4℃ for 2 hrs (stage 3). After processing of acorn *mook*, acorn *mook* ethanol extract was prepared by the same method used for acorn ethanol extraction.

7. ROS Production

Cellular ROS generation was measured using 2',7'-dichlorofluorescein diacertate (DCF-DA, Sigma) as described before (Kim HJ *et al* 2015). AEtOH or AMEtOH-treated (0, 100, 300, and 500 μg/mL) cells were seeded into 96-well plates at density of 2×10^5 cells/mL and cultured. DCF-DA was added to each well and incubated at 37℃ for 45 min.

Fig. 1. Acorn *mook* and ingredients.
Ingredients of acorn *mook* (AM) were 40 g of dried whole acorn (*Quercus acutissima* Carr.) powder, 60 g of mung bean starch, 3 g of salt, and 900 mL of water.

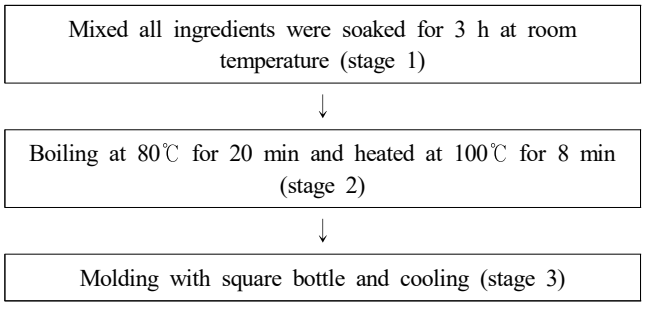

Fig. 2. Acorn *mook* making process.
Acorn *mook* was prepared as shown in Fig. 1.

Cells were then washed with phosphate buffered saline (PBS) twice. The absorbance of each well was then measured at 495 nm/527 nm using a microplate reader.

8. Oil-red O Straining

For qualitative and quantitative analysis of intracellular lipids, oil red O staining was performed as described previously (Thomson *et al* 2004). Briefly, differentiated 3T3-L1 adipocytes treated with AEtOH or AMEtOH at various concentrations (0, 100, 300, and 500 μg/mL) were washed twice with PBS (P6148, Sigma) and fixed with 200 μL of 4% (w/v) formaldehyde at 4℃ for 1 h. After removing fixing solution, cells were stained with 500 μL of Oil-red O solution (60% 2-propanol and 40% water, filtered with a 0.45 μm filter) for 30 min. After removing the staining solution, plates were rinsed with water three times and dried. Stained lipid droplets appeared red under a light microscope equipped with a chargecoupled device (CCD) camera (BX-51, Olympus, Tokyo, Japan). Oil red O-positive cells were also measured with a microplate reader at wavelength of 500 nm.

9. Statistical Analysis

Results are represented as means±standard deviations. To determine differences between groups, data were analyzed by one-way analysis of variance (one-way ANOVA) with Statistical Package for Social Sciences (SPSS) version 21.0 (SPSS Inc., Chicago, IL, USA). Statistical significance was considered at $p<0.05$.

RESULTS AND DISCUSSION

1. Antioxidant Activities of AEtOH

Total polyphenol, total flavonoid content, DPPH radical scavenging activity, ABTS radical scavenging activity, and ORAC of acorn ethanol extract (AEtOH) were showed to be 476.44±14.70 mg TAE/g, 102.91±13.14 mg QE/g, 88.36%, 75.04%, and 81.51 μM TE/g, respectively (Table 1). Polyphenols is a chemical compound found in plants, such as catechin, resveratrol and quercetin. Flavonoids and isoflavones are also a kind of polyphenols. These polyphenols have antioxidant effects that scavenger reactive oxygen in our body, preventing aging. It is also known to prevent heart disease with anticancer activity and with potential efficacy to modulate obesity and related disorders (Mattera R *et al* 2017).

Table 1. Total polyphenol and total flavonoid contents of AEtOH

Variables	AEtOH[5)]
Total polyphenol contents (mg TAE[1)]/g)	476.44±14.70[6)]
Total flavonoid contents (mg QE[2)]/g)	102.91±13.14
DPPH radical scavenging activity (%)	88.36±2.21
ABTS radical scavenging activity (%)	75.04±1.97
ORAC[3)] (μM TE[4)]/g)	81.51±1.08

1) TAE: Tannic acid equivalent.
2) QE: Quercetin equivalent.
3) ORAC: Oxygen radical absorbance capacity.
4) TE: Trolox equivalent.
5) AEtOH: Ethanol extract of acorn powder removed outer peel.
6) Data are presented as mean±S.D. (n=3).

Therefore, acorn is rich in phenolic compounds and our results suggest that AEtOH has antioxidant properties.

2. Cell Cytotoxicity of AEtOH

To determine cell cytotoxicity after exposure to AEtOH, extract was used to treat cells at different concentrations (0, 100, 300, and 500 μg/mL) for 24 h. The cell cytotoxicity was 94% or higher under all conditions (Fig. 3). This shows that AEtOH has no cytotoxic effect on 3T3-L1 cells.

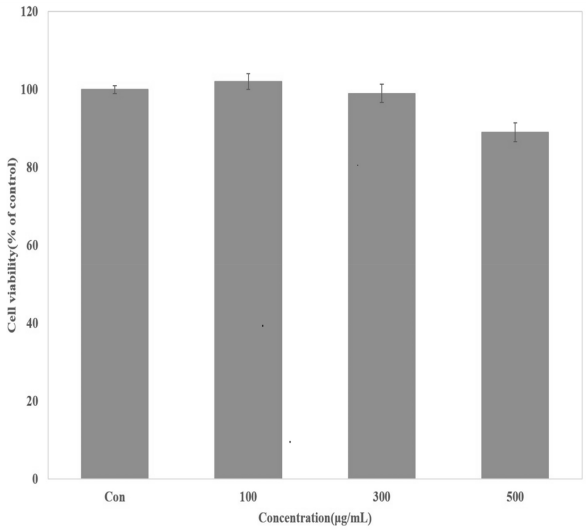

Fig. 3. Effect of AEtOH extracts on cell viability in 3T3-L1 cells.

3T3-L1 cells were treated with AEtOH extracts at different concentrations (10, 300, and 500 μg/mL) for 24 h. Cell viability was tested using the MTT assay.

3. Inhibitory Effect of AEtOH on C/EBPα, C/EBPβ, PPARγ, and aP2 Protein Expression in Differentiated 3T3-L1 Preadipocytes

Adipose tissue is an endocrine organ that secretes many cytokinenes and regulates preadipose cell growth, adipose differentiation and lipogenesis in fat cells (Khalipourfarshbafi M 2019). During adipocyte differentiation, fibroblast-like preadipocytes differentiate into lipid accumulation and insulin-responsive mature adipocytes. Increased adipocyte cell number or the size of adipocytes leads to fat accumulation (Rizzatti V *et al* 2013). Adipocyte differentiation rely on coordinated regulation of transcription factors and PPARγ is seemed to a critical transcription factor in adipogenesis. It can induce the expression of C/EBPα. C/EBPα is another critical transcription factor in adipogenesis (Rosen ED 2002). This is consistent with previous finding showing that acorn methanol extracts can inhibit mRNA expression levels of C/EBPα, C/EBPβ, and PPARγ in differentiated 3T3-L1 cells (EL-Jack AK 1999). Therefore, in order to examine the effects of acorn ethanol extracts (AEtOH) on the expression C/EBPα, C/EBPβ, PPARγ,

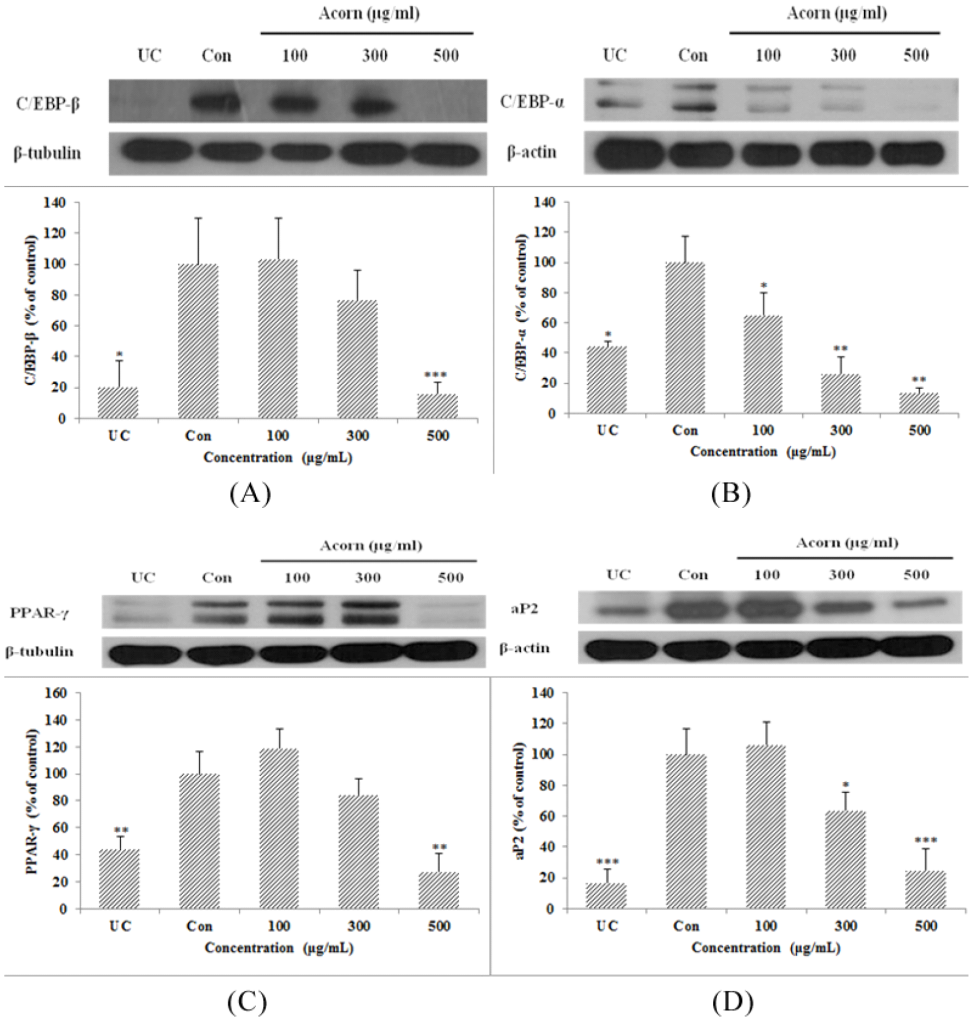

Fig. 4. Effect of AEtOH on the expression of C/EBP-β, C/EBP-α, PPAR-γ, and aP2 protein in 3T3-L1 cells.

3T3-L1 cells were treated of different concentrations of AEtOH (0, 100, 300, and 500 μg/mL) for 8 days. From whole cell lysate, protein was resolved by 12% SDS-PAGE for determination of C/EBP-β, C/EBP-α, PPAR-γ, and aP2 expression. β-actin expression is shown as a loading control. The results are reported as the mean±S.D. of three independent experiment (n=3).

UC: undifferentiated 3T3-L1 preadipocyte cell, Con: differentiated 3T3-L1 preadipocyte cells without treatment of acorn ethanol extract. (A) CCAAT/enhancer binding protein peroxisome-β (C/EBP-β), (B) CAAT/enhancer binding protein peroxisome-α (C/EBP-α), (C) Peroxisome proliferator-activated receptor-γ (PPAR-γ), (D) Adipocyte protein 2 (aP2).

Significantly different with compared to control (Con) by student's *t*-test ($p<0.05$).

and aP2, 3T3-L1 pre-adipocytes, AEtOH was treated at different (0, 100, 300, and 500 μg/mL) concentrations. Results showed that protein expression levels of C/EBPα, C/EBPβ, PPARγ, and aP2 in adipocytes treated with AEtOH (500 μg/mL) were significantly decreased compared with those in control cells (Fig. 4).

4. ROS Production and Total Lipid Accumulation in 3T3-L1 Cells

Moreover, I evaluated the influence of acorn *mook* ethanol extracts (AMEtOH) of ROS production and lipid accumulation. Reactive oxygen species (ROS) are important regulators of eukaryotic signal transduction. They are also dangerous because they can oxidize proteins, lipids, and DNA, thus impairing the function of these molecules (Rhee SG 2006). Increased oxidative stress is a key factor that causes obesity-related metabolic complications. In other words, when the oxidative stress is increased in the adipose tissue, it is induces an inflammatory signals, adipokine dysregulation, and metabolic diseases and thus it is very significant to inhibit the production of reactive oxygen species (Maggei *et al* 2018). And the main roles of adipocytes in vertebrates include triglycerides or releasing free fatty acids in response to change in energy demands (Keaney JF *et al* 2003). Therefore the effects of different concentrations of AEtOH and AMEtOH on 3T3-L1 cells on ROS production and fat accumulation were examined.

Results were shown in Fig. 5. ROS production in 3T3-L1 cells treated with AMEtOH at various concentrations (0, 100, 300, and 500 μg/mL) was significantly (p<0.05) inhibited (by 6%, 25%, and 35% at 100, 300, and 500 μg/mL, respectively) compared to that treated with AEtOH. The inhibitory effects of AMEtOH (0, 100, 300, and 500 μg/mL) on adipogenesis of 3T3-L1 cells compared to those of AEtOH were visualized using Oil-red O staining (Fig. 6). The level of lipid accumulation in AMEtOH-treated 3T3-L1 cells was significantly (p<0.05) suppressed (by 20.3% following treatment with 300 μg/mL of AMEtOH and by 37.3% following treatment with 500 μg/mL of AMEtOH) compared to that in AEtOH-treated cells.

CONCLUSION

Our study showed that acorn *mook* ethanol extract suppress lipid accumulation in 3T3-L1 cell. These results suggest that

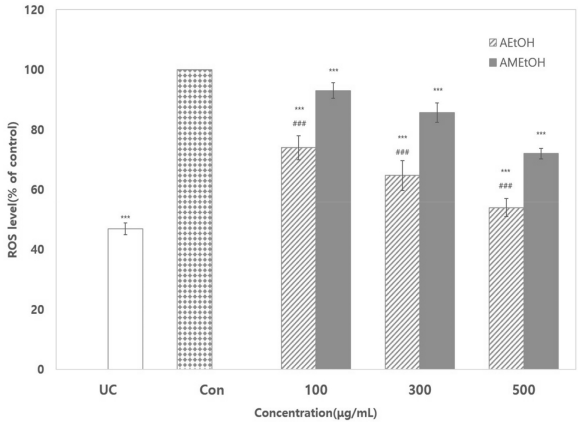

Fig. 5. Effect of AEtOH and AMEtOH on ROS production in 3T3-L1 cells.

The results are reported as the mean±S.D. of three independent experiment (n=3). Values with different super-script letters are significantly different as determined by student's *t*-test.
Significantly different with compared to control (Con) by student's *t*-test ($^{*}p$<0.01, $^{*}p$<0.001).
significantly different between AEtOH and AMEtOH by student's *t*-test ($^{\#}p$<0.01, $^{\#}p$<0.001).
UC: undifferentiated 3T3-L1 cell, Con: differentiated 3T3-L1 cells, AEtOH: acorn ethanol extract, AMEtOH: acorn *mook* ethanol extract.

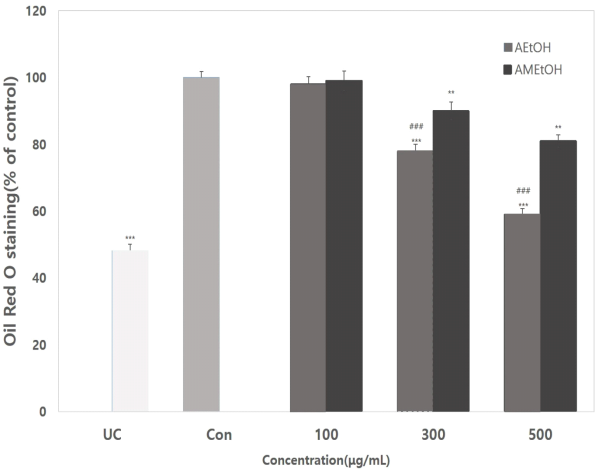

Fig. 6. Effect of AEtOH and AMEtOH on lipid accumulation in 3T3-L1 cells.

The results are reported as the mean±S.D. of three independent experiment (n=3). Values with different super-script letters are significantly different as determined by student's *t*-test.
Significantly different with compared to control (Con) by student's *t*-test (p<0.05).
significantly different between AEtOH and AMEtOH by student's *t*-test ($^{\#}p$<0.01, $^{\#}p$<0.001).
UC: undifferentiated 3T3-L1 cell, Con: differentiated 3T3-L1 cells without treatment with acorn ethanol extract.

acorn *mook* could be a potent food for obeisty prevention.

REFERENCES

Auddy B, Ferreira M, Blasina F, Lafon L, Arredondo F, Dajas F, Tripathi PC, Seal T, Mukherjee B (2003) Screening of antioxidant activity of three Indian medicinal plants, traditionally used for the management of neurodegenerative diseases. J Ethnopharmacol 84(2-3): 131-138.

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72(1-2): 248-254.

Chukir T, Shukla AP, Saunders KH, Aronne LJ (2018) Pharmacotherapy for obesity in individuals with type 2 diabetes. Expert Opin Pharmacother 19(3): 223-231.

El-Jack AK, Hamm JK, Pilch PF, Farmer SR (1999) Reconstitution of insulin-sensitive glucose transport in fibroblasts requires expression of both PPARgamma and C/EBPα. J Biol Chem 274(12): 7946-51.

GutiFinger T, Letan A (1974) Quantitative changes in some unsaponifiable components of soya bean oil due to refining. J Sci Food Agric 25(9): 1143-1147.

Iyer A, Fairlie DP, Prins JB, Hammock BD, Brown L (2010) Inflammatory lipid mediators in adipocyte function and obesity. Nat Rev Endocrinol 6(2): 71-82.

Kim HJ, Park GS, Kim Wk, Son SU, Sung JH (2015) Functional regulation of adipose-derived stem cells by PDGF-D. Stem Cells 33(2): 542-556.

Kim JO, Lee MJ (1976) Studies on some physico-chemical properties of the acorn starch. Korean J Food Sci Technol 8(4): 230-235.

Keaney JF, Larson MG, Vasan RS, Wilson PW, Lipinska I, Corey D, Massaro JM, Sutherland P, Vita JA, Benjamin EJ (2003) Obesity and systemic oxidative stress: Clinical correlates of oxidative stress in the framingham study. Arterioscler Thromb Vasc Biol 23(3): 434-439.

Khalilpourfarshbafi M, Gholami K, Murugan DD, Abdul Sattar MZ, Abdullah NA (2019) Differential effects of dietary flavonoids on adipogenesis. Eur J Nutr 58(1): 5-25.

Korea Health Statistics 2016: Korea National Health and Nutrition Examination Survey (KNHANES Ⅶ-1), Korea Centers for Disease Control and Prevention (KCDC), No. 11-1351159-000027-10.

Maggie SB, Derek KH, Jessica NK, Kelsey AS, Mario K (2018) Contribution of adipose tissue inflammation to the development of type 2 diabetes mellitus. Compr Physiol 9(1): 1-58.

Mattera R, Benvenuto M, Giganti MG, Tresoldi I, Pluchinotta FR, Bergante S, Tettamanti GM, Masuelli L, Manzari V, Modesti A, Bei R (2017) Effects of polyphenols on oxidative stress-mediated injury in cardiomyocytes. Nutrients 9(5): 523-566.

Moon HR, Chung MJ, Park JW, Cho SM, Cho DJ, Kim SM, Chun MH, Kim IB, Kim SO, Jang SJ, Park YI (2013) Antiasthma effects through anti-inflammatory action of acorn (*Quercus acutissma* Carr.) *in vitro* and *in vivo*. J Food Biochem 37(1): 108-118.

Moreno MI, Isla MI, Sampietro AR, Vattuone MA (2000) Comparison of the free radical scavenging activity of propolis from several regions of Argentina. J Ethnopharmacol 71(1-2): 109-114.

Murphy N, Jenab M, Gunter MJ (2018) Adiposity and gastrointestinal cancers: Epidemiology, mechanisms and future directions. Nat Rev Gastroenterol Hepatol 15(11): 659-670.

Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, Mullany EC, Biryukov S, Abbafati C, Abera SF (2014) Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: A systematic analysis for the global burden of disease study. J Lancet 384(9945): 766-781.

Prior RL, Hoang H, Gu L, Wu X, Bacchiocca M, Howard L, Hampsch-Woodill M, Huang D, Ou B, Jacob R (2003) Assay for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORAC (FL))) of plasma and other biological and food samples. J Agric Food Chem 51(11): 3273-3279.

Reho JJ, Rahmouni K (2017) Oxidative and inflammatory signals in obesity-associated vascular abnormalities. Clin Sci (Lond) 131(14): 1689-1700.

Rhee SG (2006) Cell signaling. H_2O_2, a necessary evil for cell signaling. Science 312(5782): 1882-1883.

Rizzatti V, Boschi F, Pedrotti M, Zoico E, Sbarbati A, Zamboni M (2013) Lipid droplets characterization in adipocyte differentiated 3T3-L1 cells: Size and optical density distribution. Eur J Histochem 57(3): e24.

Romacho T, Elsen M, Röhrborn D, Eckel J (2014) Adipose

tissue and its role in organ crosstalk. Acta Physiologica 210(4): 733-753.

Rosen ED, Hsu CH, Wang X, Sakai S, Freeman MW, Gonzalez FJ, Bruce MS (2002) C/EBPalpha induces adipogenesis through PPARgamma: A unified pathway. Genes Dev 16(1): 22-26.

Sung IS, Park EM, Lee MK, Han EK, Jang JY, Cho SY (1997) Effect of acorn extracts on the antioxidative enzyme system. J Korean Soc Food Sci Nut 26(3): 494-500.

Thompson GM, Trainor D, Biswas C, LaCerte C, Berger JP, Kelly LJ (2004) A high capacity assay for PPARgamma ligand regulation of endogenous aP2 expression in 3T3-L1 cells. Anal Biochem 330(1): 21-28.

White UA, Tchoukalova YD (2014) Adipose stem cells and adipogenesis. pp 5-32. In: Adipose tissue and adipokines in health and disease. Humana Press, New York, USA.

Date Received Jul. 22, 2019
Date Revised Nov. 2, 2019
Date Accepted Nov. 4, 2019