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마이크로파 처리 유무에 따른 우엉뿌리의 항비만 효과 비교 연구

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The comparative study on the anti-obesity effect of *Arctium lappa* L. roots with and without microwave processing

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ABSTRACT

Objective : The present study comparatively analyzed the anti-obesity effect of *Arctium lappa* L. roots with and without microwave processing.

Methods : Four HFD groups except for the Normal group (n=8) were allocated: Control, microwave-processed dried *Arctium lappa* L. roots (MAL) extract 400 mg/kg/d (MAL400), MAL extract 800 mg/kg/d (MAL800), and the dried *Arctium lappa* L. roots (DAL) extract 800 mg/kg/d (DAL800). The efficacy of MAL and DAL was confirmed in terms of the serum biochemical index, protein expressions related to the synthesis of triglyceride (TG) and total cholesterol (TC) and β-oxidation, and histopathological staining.

Results : Both MAL and DAL treatments significantly reduced final body weight and body weight gain. MAL800 treatment significantly reduced serum TG, TC, low-density lipoprotein cholesterol, and leptin levels, but the serum high-density lipoprotein cholesterol and adiponectin concentrations were dramatically increased. In particular, leptin in the MAL800 group was reduced by 14.1% compared with the DAL800 group. Moreover, the MAL800 treatment showed an effect superior to the DAL800 treatment in the reduction of serum TC, the sirtuin 1 (Sirt1) activation, and the inhibition of 3-hydroxy-3-methyl glutaryl coenzyme A reductase (HMGCR) gene expression. In particular, unlike the DAL800 treatment, MAL treatment significantly led to the activation of peroxisome proliferator-activated receptor alpha (PPARα). Subsequently, PPARα meaningfully regulated downstream proteins associated with β-oxidation.

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Conclusion : These findings suggest that *Arctium lappa* L. roots with microwave processing effectively ameliorate obesity through the regulation of leptin and TC and the promotion of β-oxidation compared with *Arctium lappa* L. roots without microwave processing.

Key words : *Arctium lappa* L. roots, microwave processing, high-fat diet, leptin, total cholesterol, anti-obesity

Ⅰ. Introduction

A number of studies, including meta-analysis, have suggested an association between obesity and COVID-19. COVID-19 has been spread rapidly around the world, mainly in regions with an alarmingly high prevalence of obesity[1,2]. Therefore, the management of obesity, one of the major risk factors for the development of severe COVID-19, will present a new challenge for the health care system[3]. At the present, the obese population leads to nearly a third of the world population and also showed an increase in obesity in all ages[4]. The enormous medical care costs caused by the rising prevalence of obesity already have been struggling in many countries[5]. Obesity is closely associated with elevated risks of not only cardiovascular disease, respiratory disorders, type 2 diabetes mellitus, arthritis, and cancer but also mental health such as depression[6,7]. Thereby, obesity can affect a decline in both quality of life and healthy life expectancy[8]. Currently, the following anti-obesity medications have been approved by the United States Food and Drug Administration (FDA): orlistat, phentermine/topiramate combination, liraglutide, and naltrexone/bupropion combination. However, these drugs have serious adverse effects in some individuals, such as abdominal pain, diarrhea, oily stools, fecal spotting, cholestatic hepatitis, subacute liver failure, and cholelithiasis. Moreover, they are quite expensive to take a long term[9,10]. Accordingly, studies are needed on emerging drug targets such as herbal remedies, which is with few side effects and equally efficacious compounds.

Arctium lappa L., commonly known as burdock, is one of the plants widely used for medical value in Asia, especially in Korea, China, and Japan[11], and in particular its roots are used as popular edible vegetables in many countries[12]. Numerous studies have reported possessing various pharmacological effects, including antioxidant, anti-inflammatory, hepatoprotective, anti-allergy, and anti-cancer[13-15]. Moreover, Ha et al. suggested a combination of aquatic exercise plus intake of burdock root water extract (100 mL plastic packs 3 times a day; total 300 mL) for 16 weeks enhanced significantly fat-related body composition parameters such as triglyceride and HDL-cholesterol levels[16]. Gao Y et al. suggested that total lignans driven from the dried ripe fruit of *Arctium lappa* L. exerts both weight loss and hypoglycemic effects in KKAy mice[17]. In a previous study, Chen reported that the clinical use of its fruit showed satisfactory efficacy in patients who suffered from diabetes[18]. Although several such studies, the anti-obesity effect and the underlying mechanism of *Arctium lappa* L. roots according to microwave processing have not been well elucidated.

Herbal medicines sometimes undergo various processing ways to reduce toxicity and ensure safety and enhance their therapeutic effects. Also, it prevents changes in the medicinal properties of herbal medicines to enable long-term storage A representative example of such a method may be steaming and drying[19-22]. In particular, when microwave vacuum drying, herbs can be dried for a short time and possess lower moisture contents for a safe, extended shelf-life, moreover, the bioactive compounds are increased[23-25].

To gain insight into the anti-obesity effect of microwave vacuum drying-processed Great Burdock, we evaluated in comparison with commonly drying-processed Great burdock in an obese mice model induced by a high-fat diet (HFD).

Ⅱ. Materials and Methods

1. Materials

Sirtuin 1 (Sirt1, SC-15404), peroxisome proliferator-activated receptor α (PPARα, SC-9000), sterol regulatory element-binding protein-1 (SREBP-1, SC-13551), stearoyl-CoA desaturase 1 (SCD1, SC-515844), sterol regulatory element-binding protein-2 (SREBP-2, SC-13552), 3-hydroxy-3-methyl glutaryl coenzyme A reductase (HMGCR, SC-33827), mitochondrial uncoupling

protein 2 (UCP2, SC-6525), histone (SC-8030) and β-actin (SC-47778) were purchased from Santa Cruz Biotechnology, Inc. (Dallas, TX, United States). AMPKα (#2532), phospho-AMPKα (#2531), acetyl-CoA carboxylase α (ACCα, SC-137104), p-ACCα (SC-271965) were purchased from Cell Signaling Technology, Inc. (Danvers, MA, United States). Carnitine palmitoyltransferase 1A (CPT1A, ab53532) was purchased from Abcam (Cambridge, United Kingdom). Enhanced chemiluminescence (ECL) western blotting detection reagents and nitrocellulose membranes were supplied by GE Healthcare (Buckinghamshire, United Kingdom). Goat anti-rabbit and goat anti-mouse immunoglobulin G horseradish peroxidase-conjugated secondary antibodies were purchased from GeneTex, Inc. (Irvine, CA, United States). Chlorogenic acid (C3828) was obtained from Sigma-Aldrich (St Louis, MO, United States).

2. Plant materials

Arctium lappa L. roots grew in Jinju, Korea was processed with a 1200W microwave for 15 min using a microwave vacuum dryer V-1400 (UNION Tech co., Ltd. Asan, Korea). Herein, the dried *Arctium lappa* L. roots (DAL) was conducted in the same procedure except for the microwave. Subsequently, it was dried with hot air at 60℃ for 24 hours using a drying oven HK-DO135F (HANKUK S&I, Hwaseong, Korea). When the moisture content was less than 5% by weight, the root of *Arctium lappa* L. was boiled with 10 folds of water at 95℃ for 4 hours. In addition, it is removed impurities using 1 μm filter paper. To obtain each yield, after extracting DAL and microwave-processed dried *Arctium lappa* L. roots (MAL) and then was concentrated to 10 Brix. Brix was measured using a Brix refractometer (MSC Industrial Supply Co., NY, USA). The yield of DAL is 36.5% and MAL is 36.9%.

3. Analyses of chlorogenic acid

Each sample (1 g) was well dissolved through sonicating for 30 minutes in 50 mL of 50% methanol, and then filtered through a 0.45 μm membrane filter and used for high-performance liquid chromatography (HPLC) analyses. We injected a 10 μL sample into the Agilent 1200 series HPLC system (Agilent Technologies, Palo Alto, CA, United States) using the Agilent Eclipse Plus C18 column (4.6 mm × 150 mm, 5 μm). The mobile phase composition was as follows: solvent A (water, 0.1% formic acid) and solvent B (acetonitrile). The gradient conditions were as follows: 5%B (0-2 min) → 5-60%B (2-10 min) → 60%B (10-12 min) → 60-5%B (12-14 min) → 5%B (14-15 min). The flow rate was 1.0 mL/min with ultraviolet (UV) absorption monitoring at 320 nm. The peak of chlorogenic acid was assigned by comparing the retention time and UV spectra of authentic standards. Chlorogenic acid was detected as a major compound from the chromatogram of the extract. A representative HPLC chromatogram is illustrated in Figure 1. Quantification of chlorogenic acid in the extract was performed by peak area measurement.

4. Experimental animals and treatment

Male-male C57BL/6J mice aged 4 weeks (20-25 g) were purchased from DBL Co., Ltd. (Eumseong, Korea). After adaptation (1 wk), all mice except the normal diet-fed mice (n = 8) were provided 60% of calories from fat (HFD Diet 12492; Research Diets, Inc., New Brunswick, NJ, USA).Thereafter, HFD-fed obese mice (n = 32) were randomly divided into the following four groups (n = 8 each): control (HFD control group (Control), MAL extract 400 mg/kg/d (MAL400), MAL extract 800 mg/kg/d (MAL800), and DAL extract 800 mg/kg/d (MAL800). The Normal and HFD control groups were given water using a stomach tube, while the drug treatment groups were orally administered DAL or MAL daily using a stomach tube for the following 6 weeks. At autopsy, mice were anesthetized with Isotroy inhalation anesthesia (induction, 4% isoflurane; maintenance, 2% isoflurane) for 5 min. Subsequently, blood was drawn from the heart puncture. The tissues (liver and epididymal adipose) were promptly stored at −80℃. All procedures were performed in accordance with the Guidelines for the Institutional Animal Care and Use Committee of the Daegu Haany University, Korea (Approval No. DHU 2022-007).

5. Biochemical analyses and metabolic measurements

To obtain the serum, the blood was centrifuged at 4000 rpm for 10 min at 4℃. The serum triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), glutamic oxaloacetic transaminase (GOT), and glutamic pyruvate transaminase (GPT) levels were measured as described previously[26,27]. Serum samples were measured for adipokine (leptin and adiponectin) using the commercial kits (Mouse Leptin ELISA Kit and Mouse Adiponectin, Koma Biotech, Seoul, Korea) following the manufacturer's instructions.

6. Tissues staining of liver and epididymal WAT tissues

A microscopic examination was performed to evaluate histological changes in the liver tissue. The fixed liver tissues in 10% neutral-buffered formalin embedded in paraffin and then stained using hematoxylin and eosin (H&E) for the morphological changes. Moreover, the effects of MAL and DAL treatment on lipid accumulation in the liver of HFD-fed mice were conducted with Oil Red O staining. A more detailed description is in the previous paper[26,27].

7. Western blotting

Liver tissues were well homogenized using buffer A. Buffer A consisted of 0.1 mM EDTA, 10 mM HEPES (pH 7.8), 0.1 mM phenylmethylsulfonyl fluoride, 10 mM KCl, 1 mM DTT, 2 mM $MgCl_2$, and 1250 μL protease inhibitor cocktail (Wako). The homogenates were incubated at 4℃ for 20 min and were mixed with 10% NP-40. After centrifugation (12,000 rpm at 4℃ for 2 min) using the Eppendorf 5415R centrifuge (Eppendorf AG, Hamburg, Germany), the supernatant was collected as the cytosol sample. Then, the lysates were suspended with 20 mL ice-cold lysis buffer C containing 50 mM HEPES (pH 7.8), 50 mM KCl, 300 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 1% (v/v) glycerol, 0.1 mM phenylmethylsulfonyl fluoride, and 100 μL protease inhibitor cocktail and incubated at 4℃ for 30 min. Samples containing 10-12 μg protein were loaded on 8%-15% SDS-polyacrylamide electrophoresis gels and subsequently electrotransferred to a nitrocellulose membrane. Each membrane was visualized using ECL reagents of GE Healthcare (Chicago, IL, USA). The bands were detected by Sensi-Q 2000 Chemidoc (Lugen Sci Co., Ltd., Suwon, Korea). The quantification analysis of each band was analyzed by application of ATTO Densitograph Software (ATTO Corporation, Tokyo, Japan). The protein levels of groups are expressed relative to those of the Normal group.

8. Statistical analysis

The data are expressed as the mean ± standard error of the mean. Statistical comparisons were performed by one-way analysis of variance followed by the least significant difference test using SPSS software (version 26.0; IBM Corp., Armonk, NY, United States). $P < 0.05$ was considered statistically significant.

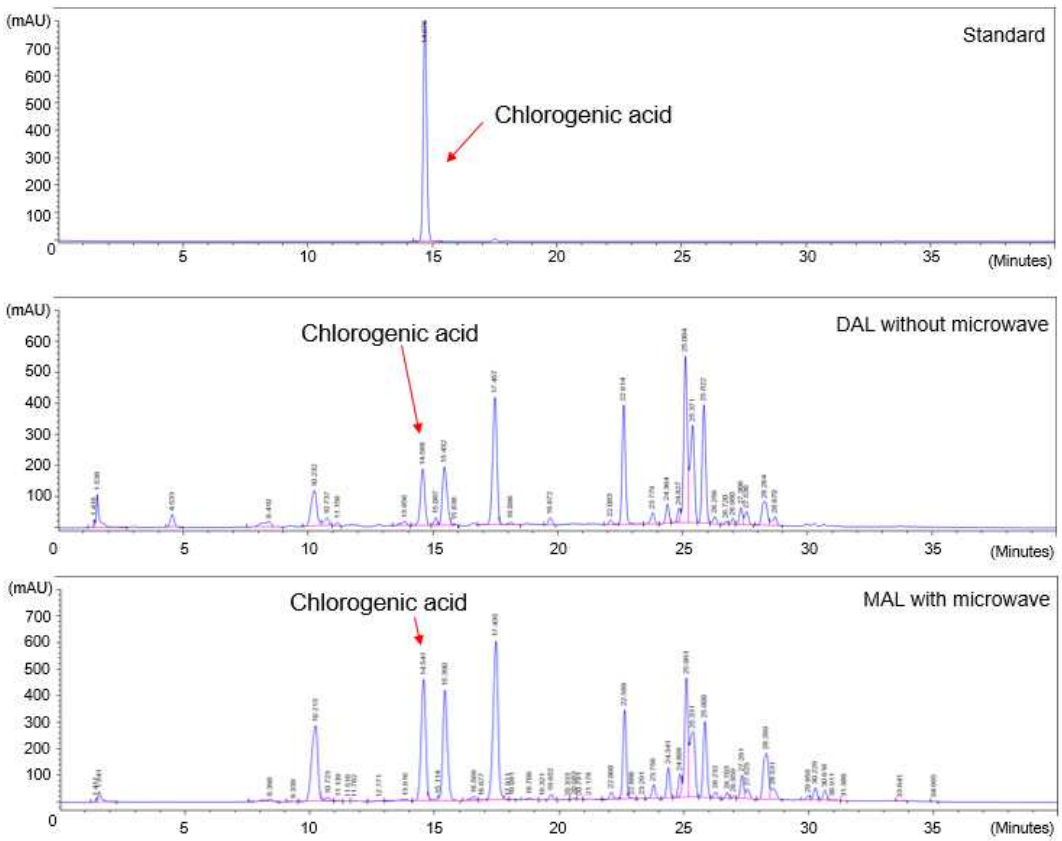

Figure 1. Analysis of chlorogenic acid in the extract of DAL and MAL at 320 nm. HPLC chromatogram of the extract of MAL and DAL. MAL, microwave-processed dried *Arctium lappa* L. roots; DAL, the dried *Arctium lappa* L. roots.

Ⅲ. Results

1. The increase of the amount of chlorogenic acid by microwave treatment

The amount of chlorogenic acid of DAL and MAL on HPLC analysis was as follows: DAL, 1.07 mg/g; MAL, 3.26 mg/g. The content of chlorogenic acid was increased 3.05 times when microwave processing.

2. Effect of MAL on body and tissue weights and food efficiency ratio

We investigated the anti-obesity effects of MAL and DAL on HFD-fed obese mice. We evaluated the difference in the body weight, epididymal fat, and liver weights (Figure 2). HFD intake dramatically increased body weight gain (p < 0.001) and significantly increased epididymal fat weight and reduced liver weight compared with the Normal group (p < 0.001). Both MAL and DAL treatments showed a significant reduction in the final body weight and body weight gain. Epididymal fat and liver weights showed a significant difference in the DAL800 group (p < 0.05). However, the effect of DAL800 didn't show a meaningful difference compared with the MAL800 group (epididymal fat, p = 0.456; liver, p = 0.106). Moreover, the food efficiency ratio (FER) in the DAL800 group was significantly lower than that of the MAL800 group (p < 0.01). For that reason, DAL treatment is considered to have appeared at a rather low level than those of MAL treatment on final body weight and body weight gain. Ultimately, the treatment of DAL and MAL showed a weight loss effect, and it is necessary to look further into what pathways such effect went through.

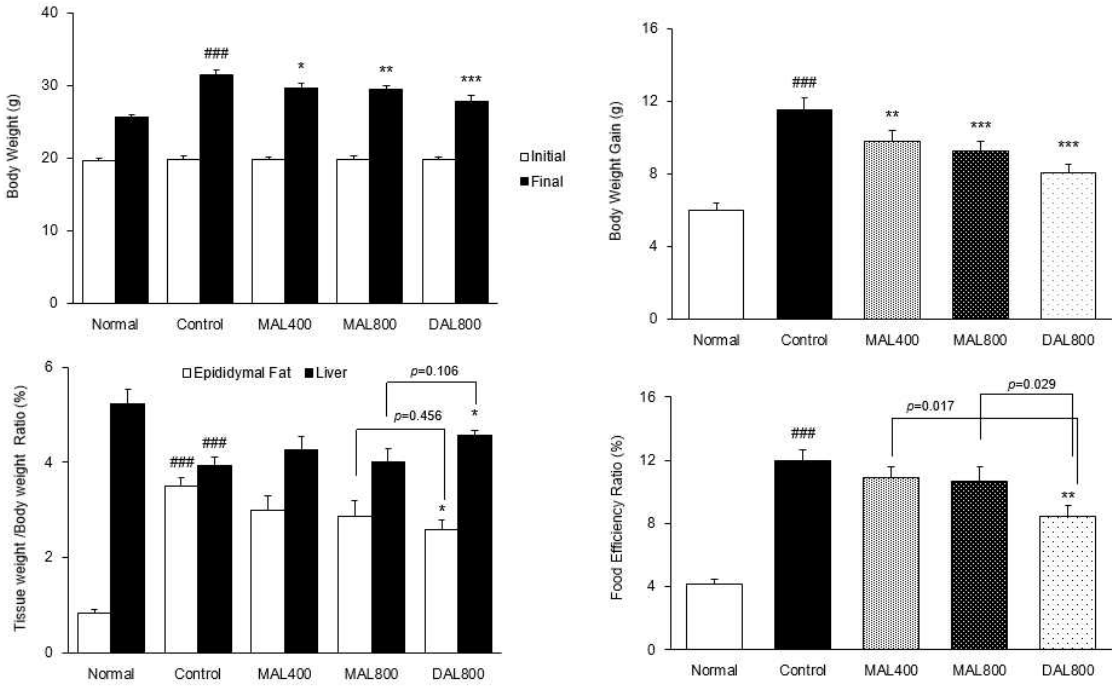

Figure 2. Effect of MAL on body weight, tissue weights ratio, and food efficiency ratio.
Mice were fed a 60% HFD and orally administered a MAL (400 and 800 mg/kg b.w) or DAL (800 mg/kg b.w) for 6 weeks. Values are expressed as mean ± SEM, n = 8. $^{###}p$ < 0.001 compared with the Normal group; $^{*}p$ < 0.05, $^{**}p$ < 0.01, $^{***}p$ < 0.001 compared with the Control group. HFD, High-fat diet; MAL, microwave-processed dried *Arctium lappa* L. roots (MAL); DAL, the dried *Arctium lappa* L. roots.

3. Effect of MAL on the serum lipid profiles and hepatic lipid accumulation

To evaluate the lipid improvement effect of MAL on HFD-induced obese mice, we measured serum lipid profiles including TG, TC, HDL-C, and LDL-C and lipid content in the liver tissues. After 6 weeks of HFD supplementation, lipid content in the liver tissues of the Control group increased, whereas MAL and DAL800 treatments were significantly alleviated (Figure. 3). Consistent with the histopathological analysis, serum biochemical levels such as TG and LDL-C by the treatment of both DAL800 and MAL were significantly attenuated whereas HDL-C significantly increased. Herein, there was no significant difference when

comparing the improvement effect between MAL800 and DAL800 (TG, p = 0.452; HDL-C, p = 0.869; LDL-C, p = 0.731). Meanwhile, the elevated level on TC noticeably reduced only MAL800 (p = 0.047). In addition, the levels of serum parameters of liver injury, including GOT and GPT, were reduced by MAL and DAL800 treatments compared with the Control group. These results considered that the improvement of HFD-induced hepatic lipid accumulation confirmed by Oil red O staining gave a significant impact on the MAL and DAL800 groups.

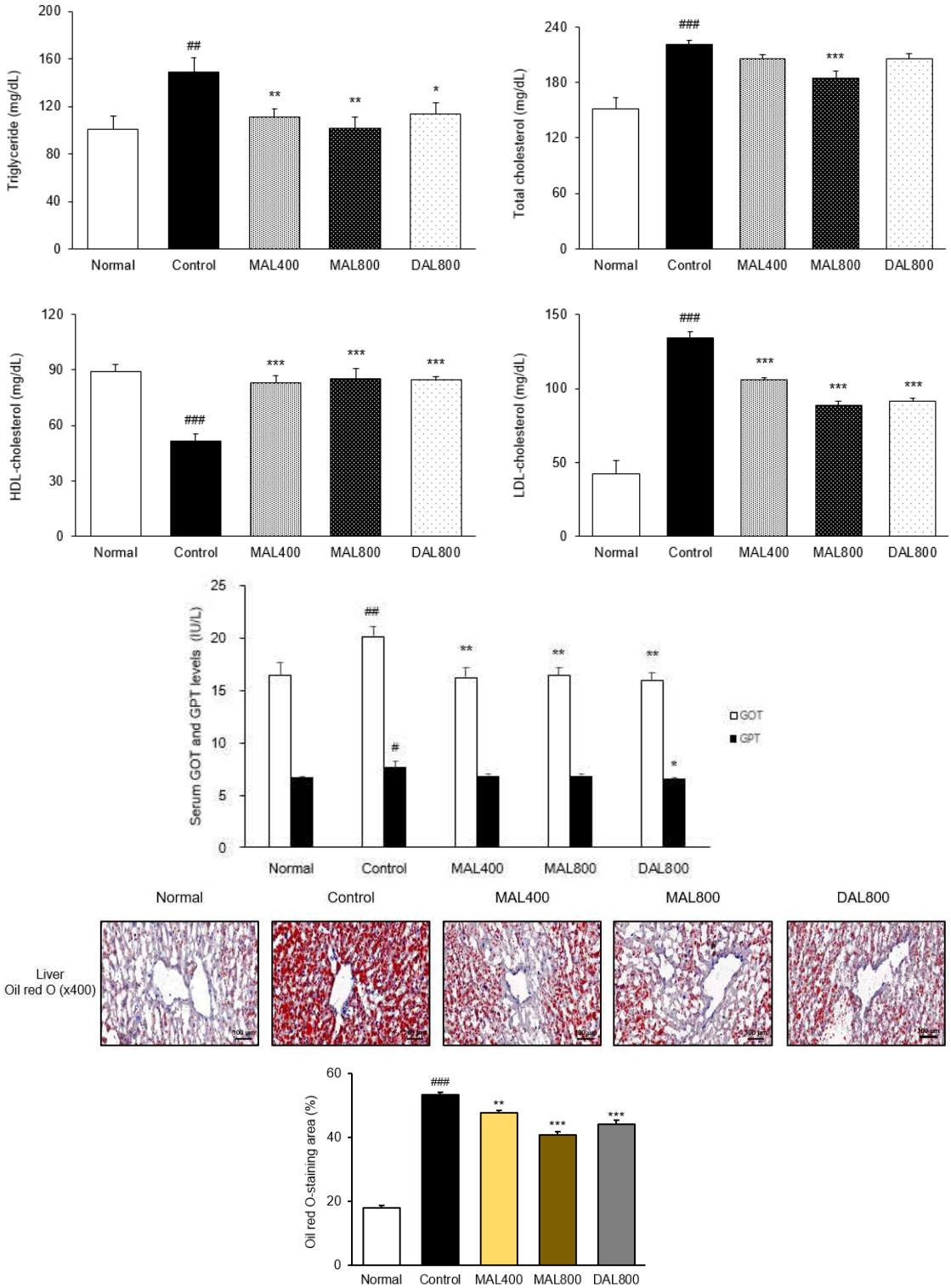

Figure 3. Effect of on serum biochemical parameters and HFD-induced hepatic lipid accumulation. Mice were fed HFD and also administrated drugs as previously indicated. Oil red O staining of hepatic tissue was evaluated under microscope (magnification, x400). Scale bar = 100 μm. Values are expressed as mean ± SEM, n = 8. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared with the Normal group; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared with the Control group.

4. Effect of MAL on the serum levels of Leptin and Adiponectin

When compared with the Normal group, HFD intake significantly stimulated the serum leptin level ($p < 0.001$) and reduced adiponectin level ($p < 0.001$). Whereas both MAL and DAL dramatically suppressed the leptin level (MAL, 65.7%; DAL, 60% vs. the Control group) and effectively elevated the adiponectin level (MAL, 23.4%; DAL, 23.7% vs. the Control group). Herein, there was no significant difference when comparing the improvement effect between DAL800 and MAL800 (leptin, $p = 0.655$; adiponectin, $p = 0.969$).

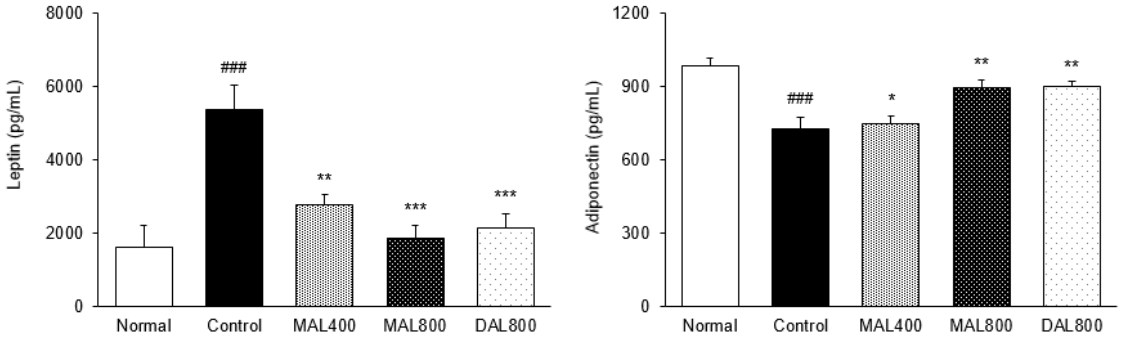

Figure 4. Effect of MAL on the serum levels of leptin, and adiponectin in the HFD-fed obese mice. Mice were fed HFD and also administrated drugs as previously indicated. Values are expressed as mean ± SEM, n = 8. $^{###}p < 0.001$ compared with the Normal group; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared with the Control group.

5. Effect of MAL on AMPK/Sirt1 activation in HFD-fed obese mice

Next, we investigated whether AMPK/Sirt1 activation promotes fatty acid oxidation or not (Figure 5). Compared with the Control group, the levels of p-AMPK (MAL400, 36.4%; MAL800, 43.5%; $p < 0.01$) and Sirt1 (MAL400, 77.7%; MAL800, 71.6%; $p < 0.01$) were markedly increased in the MAL groups. However, the DAL800 group elevated compared with the Control without a significance. When comparing the MAL800 and DAL800 groups on Sirt1 activation, the MAL800 group increased significantly compared with the DAL800 group. Moreover, ACC phosphorylation effectively increase in the only MAL800 group ($p < 0.01$). Other than that, the expression of CPT-1 in drug-treated groups markedly increased compared with the Control group. As a result, it was shown that MAL800 promotes fatty acid oxidation by effectively inhibiting the phosphorylation of ACC through AMPK/Sirt1 activation. PPARα activation in the MAL groups led to an increase in β-oxidation. Especially, the improvement effects of the MAL800 treatment are superior to those of the DAL800 treatment.

6. The effect of MAL on TG and TC synthesis in HFD-fed obese mice

The immunoblot results revealed that HFD supplementation caused an increase in the expressions of the transcription factors such as SREBP-1 and SREBP-2 (Figure 6). However, MAL administration meaningfully inhibited such elevated protein levels (SREBP-1, $p < 0.01$; SREBP-2, $p < 0.001$). The inhibition of SREBP-1 led to the reduction of lipogenic genes (ACC and SCD1) and the inhibition of SREBP-2 suppressed the activity of HMGCR. Above all, the HMGCR level by MAL800 treatment showed a significant difference compared with the DAL800 group ($p = 0.028$). These data showed that MAL treatment regulated excellently the protein expressions of both SREBP-2 and HMGCR.

7. MAL improved histological alterations in the liver and adipose tissue

As shown in Figure 7, H&E staining of epididymal WAT showed that the size of adipocytes was dramatically increased in the Control group compared with the Normal group. Furthermore, liver sections in the Normal group showed neatly arranged cells without accumulation of lipid droplets and the infiltration of inflammatory cells. However, the Control group showed myriad lipid droplets and marked inflammatory cell infiltration. In particular, the pathological condition in liver tissue of obese mice by MAL or DAL treatment was more effectively alleviated than that of the Control group.

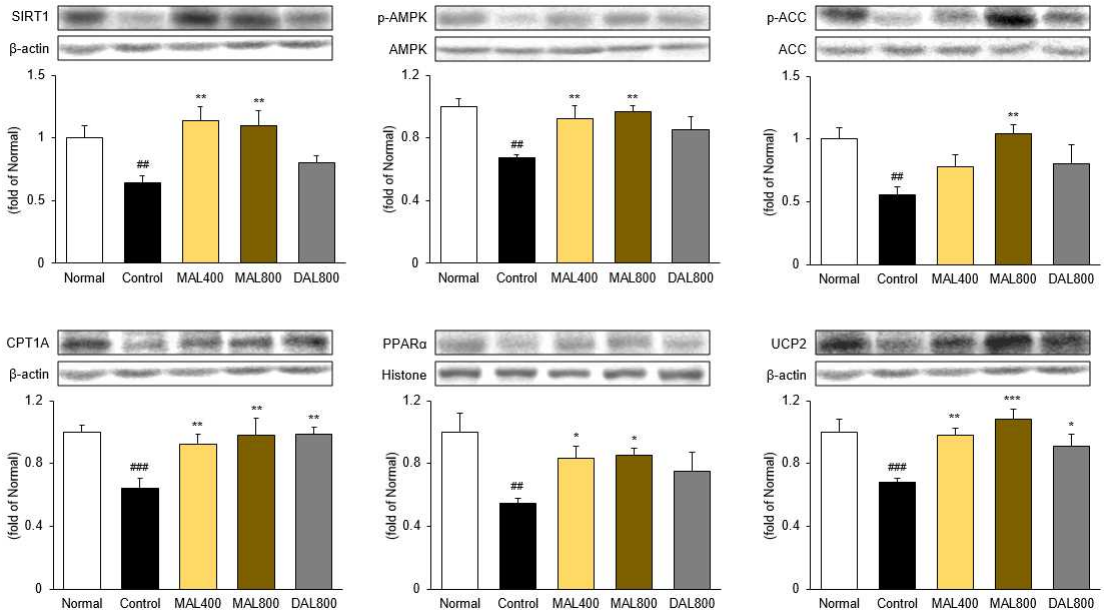

Figure 5. Effect of MAL on proteins involved in fatty acid oxidation in the HFD–fed obese mice. Mice were fed HFD and also administrated drugs as previously indicated. Values are expressed as mean ± SEM, n = 8. $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared with the Normal group; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared with the Control group.

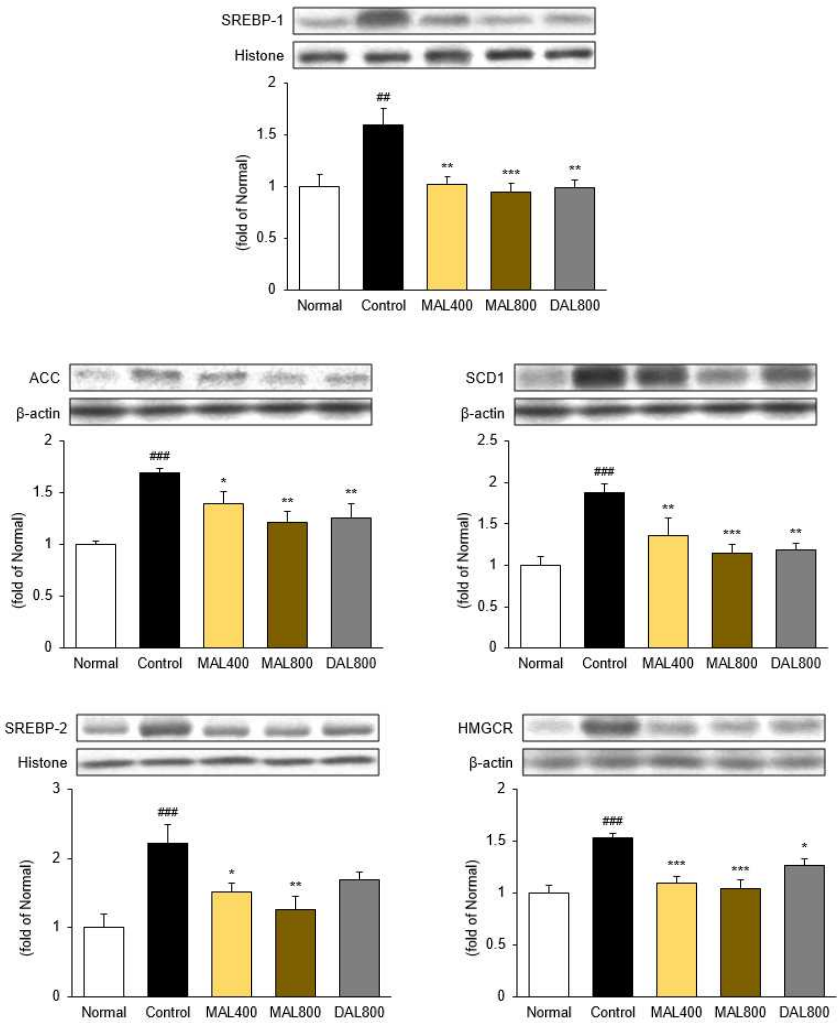

Figure 6. Effect of MAL on proteins involved in the synthesis of triglyceride (TG) and total cholesterol in the HFD–fed obese mice. Mice were fed HFD and also administrated drugs as previously indicated. Values are expressed as mean ± SEM, n = 8. $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared with the Normal group; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared with the Control group.

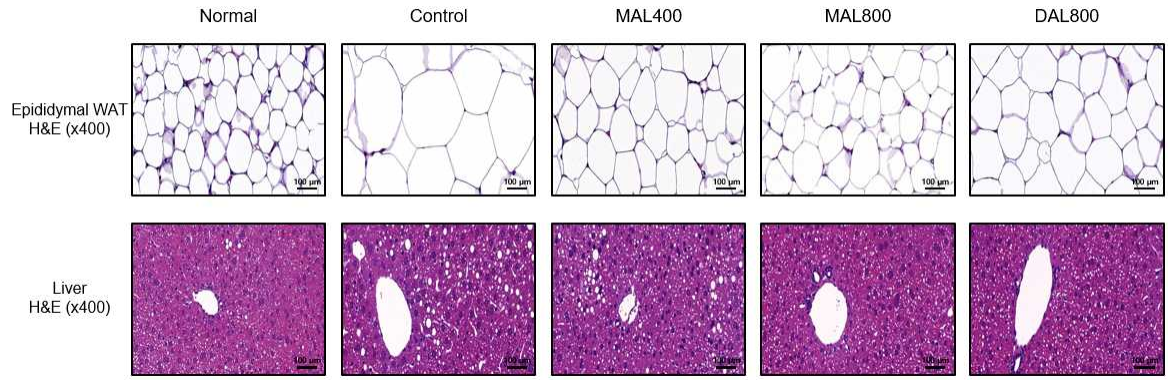

Figure 7. Effect of MAL on HFD-induced histopathological changes. Mice were fed HFD and also administrated drugs as previously indicated. Epididymal WAT and Liver tissues were observed by H&E staining under a microscope (magnification, x400). Scale bar = 100 μm.

IV. Discussion

The continuous increase in the prevalence of obesity, which is defined as the unusually excessive fat deposition is emerging as a major health problem facing the world today[28,29]. Lifestyle habits, consumption patterns, and urban development influence the prevalence of obesity. Obesity can lead to a decrease in life expectancy, the deterioration of quality of life, and an increased risk of cancer. Moreover, the health care cost for its treatment causes an economic burden[30]. Therapeutic strategies for obesity treatment include diet, lifestyle changes, medications, and surgical operations (bariatric surgery). Despite some effective and desirable results, obesity remains an unsolved problem. A safer and more effective new treatment using natural herbs seems to be an alternative strategy to overcome the previous limitations[31].

Adipose tissue, the liver, and skeletal muscle are well-known fundamental organs for the maintenance of lipid homeostasis and the metabolic regulation of fatty acids. In particular, the attention to three critical mediators including fatty acids, leptin, and adiponectin in a number of researches is focused[29]. In addition, herbal medicines are sometimes processed for enhancing their therapeutic effects[23-25]. In particular, the micro-ave process was formerly widely used in food preparation processes such as drying but is now being applied across a variety of industries, including novel medical and biosensor diagnostics[32,33]. Based on this, *Arctium lappa* L. roots with a micro-wave process were used in this experiment.

Ultimately, this study revealed both the anti-obesity effect and the underlying mechanism of *Arctium lappa* L. roots according to with and without processing as its first-attempt efforts.

A recent study reported that chlorogenic acid exerts an anti-obesity effect and modulates gut microbiota in high-fat-fed mice[34]. In addition, other studies revealed that it ameliorates obesity by preventing energy balance shift through the improvement of glucose intolerance mainly via regulating energy expenditure and food intake in obese mice[35]. Therefore, at first, the content of chlorogenic acid, a natural polyphenol, was compared through HPLC. As a result, it was confirmed that there was a 3.05 times increase in MAL compared to DAL. Based on previous reports, it is judged that this increase in the content of chlorogenic acid will lead to an anti-obesity effect.

Prolonged intake of HFD results in elevated body weight and fat mass[36]. In the current study, the Control group showed a significantly higher final body weight and body weight gain than those of the Normal group, indicating that obesity was successfully induced. Treatment with MAL and DAL800 showed a significant reduction such increase. Interestingly, the reason that DAL showed a slightly better weight loss effect than MAL was probably because of the lower DAL in feed efficiency ratio (FER). FER of DAL800 showed a significant difference compared with FER of MAL (MAL400, p = 0.017; MAL800, p = 0.029). For that reason, the DAL800 treatment showed a significant decrease compared to the Control group in the tissue weight ratio ($p < 0.05$), but did not inspire a significant difference when compared with the MAL800 treatment (Figure 2).

Increased fat induced by long-term HFD intake is associated with dyslipidemia, which is a term collectively used to refer to not only increased TG, TC, and LDL-C but also decreased HDL-C. Dyslipidemia promotes the occurrence of coronary artery disease (CAD)[37]. The

results of this study show that both the MAL and DAL800 groups improved the changes in serum lipids by reducing TG and LDL-C levels and simultaneously increasing HDL-C levels. Surprisingly, the increased TC level was alleviated significantly by only MAL800 treatment. In addition, the levels of serum parameters of liver injury, including GOT and GPT, were reduced by MAL and DAL800 treatments compared with the Control group (Figure 3). These results considered that the improvement of HFD-induced hepatic lipid accumulation confirmed by Oil red O staining had a significant impact in the MAL and DAL800 groups.

Leptin is one of the major hormones that is predominantly produced by the adipocytes, which regulates appetite and satiety[38]. High concentrations of serum leptin are found when obese and have deep relevance to an increased risk of metabolic diseases such as type 2 diabetes and vascular complications such as atherosclerosis and hypertension[39]. Unlike leptin, adiponectin is involved in regulating fatty acid breakdown and also inhibits the development of atherosclerotic plaque. In our study, leptin level was noticeably reduced in all drug-treated groups, while adiponectin significantly increased in all drug-treated groups. The MAL800 group in serum leptin reduced 14.1% compared with the DAL800 group. However, the adiponectin level showed a similar feature between the MAL800 and DAL800 groups (Figure 4).

The previously accumulated evidence suggests that AMPK plays a major role in the control of lipid metabolism in the liver[40]. AMPK activation suppresses adiposity in HFD-induced obese mice through interaction with Sirt1[41]. In this study, we confirmed that AMPK/Sirt1 protein levels were significantly reduced in the Control group compared with the Normal group, while the decreased protein levels were dramatically increased by MAL treatment whereas the DAL800 treatment showed a tendency to increase them (Figure 5). A number of studies indicated that PPARα agonists suppressed dyslipidemia *via* AMPK activation in metabolic disorders[42]. PPARα performs fundamental roles in glucose metabolism, fatty acid oxidation, and lipid homeostasis. Furthermore, the activation of PPARα reduces TG level and increases HDL-C level. Moreover, PPARα is positively correlated with CPT-1 expression, an enzyme essential for fatty acid oxidation[43]. In addition, PPAR activators promote the expression of UCP1 protein in brown adipose tissue and UCP2 protein in liver tissue. Our present study demonstrated that HFD-fed mice had markedly decreased protein expression of fatty acid oxidation markers via the AMPK/Sirt1 signaling pathway. However, the MAL800 treatment significantly reversed the reduced protein expression. Especially, the improvement effects of the MAL800 treatment are superior to those of the DAL800 treatment.

Furthermore, activation of AMPK is associated with inhibition of SREBPs activity, which are well-known as important transcription factors for TG and TC synthesis. SREBP-1 primarily regulates the genes involved in fatty acid and triglyceride synthesis, whereas SREBP-2 predominantly controls cholesterol synthesis[44]. Activation of SREBP-1, which means Ser372 phosphorylation, not only prevents its proteolytic processing but also promotes the lipogenic process. Subsequently, it leads to the up-regulation of ACC and SCD-1 and accelerates *de novo* fatty acid synthesis[45]. SREBP-2 regulates gene expression of HMGCR, which is regarded as the major rate-limiting enzyme in cholesterol biosynthesis[46]. In this study, both MAL and DAL800 treatment revealed the significant reduction of lipogenic genes such as ACC and SCD-1 through the inhibition of SREBP-1. Whereas, in the case of TC synthesis, unlike the DAL800 group, only the MAL groups showed excellent inhibitory. HMGCR in the MAL800 group was reduced by 12.5% compared with the DAL800 group (Figure 6). These results indicate that the MAL800 treatment showed a superior effect in the control of TC synthesis through inhibition of SREBP-2 compared with the DAL treatment.

Finally, as shown in Figure 7, H&E staining of epididymal WAT showed that the size of adipocytes was dramatically increased in the Control group compared with the Normal group[47]. In addition, H&E staining of the liver of the Control group showed myriad lipid droplets and marked inflammatory cell infiltration. However, these pathological changes were more effectively alleviated by MAL or DAL treatment.

Ⅴ. Conclusion

In this study, the anti-obesity effects of *Arctium lappa* L. roots following microwave treatment were confirmed in an animal model induced by a high-fat diet, and our results are as follows.

1. DAL800 is at a rather low level than those of MAL800 on final body weight and body weight gain. However, these results are considered to be due to a significantly lower feed efficiency ratio of DAL than MAL.

2. In the Oil red O staining, HFD-induced hepatic lipid accumulation gave a significant impact on the MAL and DAL800 groups. Moreover, the inhibition of fat accumulation showed similar patterns in both MAL800 and DAL800.

3. In serum biochemical analysis of lipid-related markers, MAL800 had lower TG, TC, and LDL-C than DAL800 and higher HDL-C.

4. As a result of leptin analysis, MAL800 showed a lower level than DAL800.

5. In the fatty acid oxidation via AMPK/Sirt1/ PPARα activation, MAL800 was superior to those DAL800.

6. In the TG and TC synthesis via SREBP-1 and SREBP-2, MAL800 inhibited more effectively than DAL800. Especially, MAL800 showed a marked inhibitory effect in TC synthesis than TG synthesis compared with DAL800.

In conclusion, these findings suggest that dried *Arctium lappa* L. roots with microwave processing may be helpful as a more effective treatment for obesity than just dried *Arctium lappa* L. roots.

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