Research Article

*Ulmus parvifolia* Jacq. Exhibits Antiobesity Properties and Potentially Induces Browning of White Adipose Tissue

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The bark of *Ulmus parvifolia* Jacq. (UP) was traditionally used as a diuretic and to treat intestinal inflammation. With modern evidence of the correlation of diuretics, gut inflammation, and obesity, our study has shown the antiobesity effects of the bark of UP. UP treatment reduced lipid production and adipogenic genes *in vitro*. *In vivo* studies revealed that UP 100mg/kg and UP 300mg/kg treatment significantly reduced mouse weight without reducing food intake, indicating increased energy expenditure. UP significantly reduced the weight of epididymal and subcutaneous adipose tissue and decreased liver weight. Histological analysis revealed improvement in the progression of nonalcoholic fatty liver disease and epididymal white adipose tissue hypertrophy induced by a HFD. Real-Time PCR of epididymal adipose tissue revealed significant increases of uncoupling protein-1 (UCP-1) and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) expression after UP 300mg/kg treatments. Phosphorylation of AMP-activated protein α (AMPKα) was increased, while phosphorylation of Acetyl-CoA Carboxylase (ACC) was reduced. Our findings reveal the ability of UP to reduce the occurrence of obesity through increased browning of white adipose tissue via increased AMPKα, PPARγ, PGC-1α, and UCP-1 expression.

1. Introduction

The *Ulmus* genus are elm trees found in North America [1], the Himalayas, and East Asia [2]. *Ulmus parvifolia* Jacq. (UP) which is native to Japan, Korea, and China has been reported for its antioxidant and anti-inflammatory activities [3]. The leaves of UP were traditionally used as an external dressing on wounds and ulcerous tissue [4] and as a lithotrictic agent [5]. According to the Chinese Supplement to Materia Medica (Bencao Gangmu Shiyi), the bark of UP is nontoxic and was used to treat strangury, burns, and intestinal inflammation [6]. Its bark was also used for its demulcent and lithotrictic properties other than treating cough and fever. It was also used as a diuretic [7]. Insulin resistance was said to be related to hypertension as the release of free fatty acids due to excess adipose tissue lipolysis induces various metabolic abnormalities, as well as vascular dysfunction. There was also evidence suggesting that peptides derived from adipocytes may affect arterial pressure, contributing to hypertension [8,9]. Diuretics have also been commonly used...
to treat obesity [10,11]. In obesity, adipocytes secrete proinflammatory cytokines (TNF-α and IL-6), also known as adipokines, into the circulation [12]. The ability of UP to treat burn injury and intestinal inflammation suggests its anti-inflammatory properties that could inhibit the proinflammatory state in adipose tissue. Gut anti-inflammatory agents were also used to regulate obesity-related insulin resistance [13], which suggests that UP may also be used to treat obesity as UP was traditionally used to treat intestinal inflammation. We have also reported that UP exhibits antiplatelet and antithrombotic activity [14]. Existing traditional and modern evidence and reports suggest that UP may exhibit antiobesity properties.

UP was also reported to inhibit nitric oxide production in lipopolysaccharide-treated RAW 264.7 murine macrophages [15], exhibit anticancer and antiviral properties [16], and accelerates skin wound healing [17]. However, there were no reports on the antiobesity effects of the bark extract of UP. Fat tissue is an organ that serves as a survival adaptation in humans by providing a source of energy during starvation and heat insulation in cold weather. Adipose tissue exists in two forms, white adipose tissue (WAT) and brown adipose tissue (BAT) [18]. Triglycerides are stored in WAT as lipids in unilocular white adipocytes. Excessive accumulation of WAT has been shown to lead to cardiovascular diseases [19], type 2 diabetes [20], and cancer [21]. BAT dissipates heat due to the activity of uncoupling protein-1 (UCP-1). Hence, BAT is active metabolically. UCP-1 functions through the dissipation of the proton gradient into the inner mitochondrial membrane [22]. Increasing UCP-1 expression increases the conversion of free fatty acids during heat dissipation, hence reducing the amount of triglycerides in the body. This could potentially be one target for therapeutic methods for reducing the incidence of obesity.

In this study, we investigate the antiobesity effects of ethanol extracts of the bark of UP in vitro using 3T3-L1 preadipocytes and in vivo in a model of mice fed a high-fat diet (HFD). Our study demonstrates that UP inhibits lipid preadipocytes and also inhibits diet (HFD). Our study demonstrates that UP inhibits lipid oxidation and the role of Sirtuin 1 (Sirt1) in mitochondrial biogenesis in conjunction with treatment with UP.

2. Materials and Methods

2.1. Reagents. The bark of UP was kindly provided by the Herbal Crop Research department of the Korean National Institute of Horticultural and Herbal Science (voucher number: NIHHS-0169), which is sourced from Danyang Province, Chungbuk, Republic of Korea. UP was verified by Professor Lee Seung Eun. Dulbecco’s modified Eagle Medium (DMEM), fetal bovine serum (FBS), streptomycin, and penicillin were purchased from Welgene (Daegu, Republic of Korea). Insulin, 3-isobutyl-1-methylxanthine (IBMX), indomethacin, Oil Red O, orlistat, and neutral buffered formalin were purchased from Sigma-Aldrich (St. Louis, MI, USA). TRizol reagent was purchased from Invitrogen (Carlsbad, CA, USA). Primers used in this study displayed in Table 1 were purchased from Bioneer (Daejeon, South Korea). Antibodies for p-AMPKα, AMPKα, p-ACC, and β-actin were purchased from Cell Signaling Technology (Danvers, MA, USA). Standards of (+)-catechin (product no. ES090-A) and catechin-7-O-β-D-apiofuranoside (product no. ES060-A) were purchased from Ensol Biosciences Inc. (Daejeon, Republic of Korea), both having a purity ≥95%.

2.2. Preparation of U. parvifolia. Bark of UP was collected and shredded. The bark was extracted using 70% ethanol at 80°C, filtered through filter paper (Whatman, USA), and condensed using a rotary evaporator before lyophilization to obtain the powder form of the extracts and weighed according to the desired concentrations.

2.3. UPLC-QToF MS Analysis of U. parvifolia. A UPLC system (Waters Corp., Milford, MA, USA) equipped with a binary solvent delivery system, an autosampler, and a UV detector was used. Briefly, aliquots of 2.0 μL of UP were injected into a BEH C18 column (2.1 × 100 mm × 1.7 μm) at a flow rate of 0.4 mL/min and were eluted with a chromatographic gradient consisting of two mobile phases, which are A, water containing 0.1% formic acid; B, acetonitrile containing 0.1% formic acid. A linear gradient was optimized: 0 min, 5%; 0–8 min, 5–15%; 8–11 min, 15–80%; 11–12 min, 80–100%; 12–13.3 min, 100%; and 13.4–15 min, back to 5% B. Using a negative ion mode with a capillary voltage of 2.3 kV, cone voltage of 50 V, source temperature of 110°C, and a desolvation temperature of 350°C, the quadrapole time-of-flight mass spectrometer (Q-ToF Premier™, Waters Corp., Milford, MA, USA) was operated. A reference solution of leucine-enkephalin ([M − H]+ m/z 554.2615) in the form of a spray was used as the lock mass. The full-scan data and the MS/MS spectra were collected with MassLynx software (Thermo Fisher, MA, USA).

2.4. GMS Analysis of U. parvifolia. An Agilent 7890A GC (Agilent Technologies, Santa Clara, CA, USA) with a
30 m × 0.25 mm i.d. DB-5MS column and an Agilent 5975C mass selective detector (MSD) were used to separate and quantify the constituents of UP. Samples were injected in split mode with a temperature of 250°C. The transfer line temperature was 280°C, and the ion source temperature was 230°C. The column temperature was held at an initial temperature of 70°C for 1 min and was raised to 300°C at a rate of 5°C/min and held at a final temperature of 300°C for 30 min. Helium was used as a carrier gas at a constant flow rate of 1 mL/min. Mass spectrometry was performed using the electron ionization (EI) and scan modes.

### 2.5. Cell Culture

3T3-L1 preadipocytes purchased from ATCC (Manassas, VA, USA) were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum, 100 IU/mL penicillin, and streptomycin and maintained at 5% CO₂ and 37°C. Differentiation was induced with media supplemented with insulin, indomethacin, and IBMX. Cells were allowed to differentiate for 3 days and then cultured with normal media supplemented with insulin for postdifferentiation maintenance. Cells were differentiated for a total of 10 days for consecutive experiments.

### 2.6. Oil Red O Staining

Cells were differentiated using differentiation media and simultaneously treated with 6.25, 12.5, and 25 μg/mL of UP. After 10 days, the cells were stained with Oil Red O staining. Oil Red O solution was then added to plates cultured with 3T3-L1 cells for visualization of lipids secreted by the cells under a microscope.

### 2.7. Cell Viability Assay

Viability of undifferentiated 3T3-L1 cells were assessed by using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The cells were seeded in 24-well plates for 24 h. Incubation of UP in specified concentrations was performed. Proceeding steps were carried out as previously reported [26]. Briefly, MTT was added to wells and left to incubate for 3 h. DMSO was added to each well to dissolve violet crystals of MTT and left on a rocker for 10 min. Plates were then read at 560 nm using a plate reader.

### 2.8. High-Fat Diet Induced Obesity in Mice

All experiments were approved by the Institutional Animal Care Committee of Kyungpook National University in accordance to NIH guidelines (approval number: 2018-0117). Four-week-old male ICR mice were purchased from Orient Bio (Gyeonggi-do, Republic of Korea), maintained in a 12-h light/dark controlled room with regulated temperature at 22 ± 2°C and humidity of 50 ± 10%. The mice were given access to chow and water ad libitum and allowed to acclimatize for one week, grouped in numbers of 6 with a total of 5 groups. Six mice were given normal chow, and the remaining mice were fed an HFD (D12492; Research Diets, New Brunswick, N),

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
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<tr>
<td>FAS</td>
<td>Forward</td>
<td>5'-CTGAGATCCAGAGCCTT TTGA-3'</td>
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<tr>
<td></td>
<td>Reverse</td>
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<tr>
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<td>FAM</td>
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<td>C/EBPα</td>
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<td>Reverse</td>
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<tr>
<td>Adiponectin</td>
<td>Forward</td>
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<td></td>
<td>Reverse</td>
<td>5'-GGACCAATACCCTGCTATAGGG-3'</td>
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<tr>
<td>IGF-1</td>
<td>Forward</td>
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<td></td>
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<tr>
<td>ACC</td>
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<td>5'-GTCTCAAGGTGCTGGGCTCCCTG-3'</td>
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<td>Leptin</td>
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<tr>
<td>GAPDH</td>
<td>Reverse</td>
<td>5'-CCCTGGCTTTATGCTTTTGCG-3'</td>
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**Table 1: Primer sequences for real-time PCR.**

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USA). Mouse weight, food intake, and water intake were measured weekly. After four weeks of a HFD, mice were given an oral administration of orlistat (10 mg/kg) and UP at 100 mg/kg and 300 mg/kg for an additional 8 weeks. Twenty-four hours after the final administration, mice were anesthetized and blood was collected by cardiac puncture. Organs and adipose tissue were immediately harvested, weighed, frozen, and fixed in neutral buffered formalin. The food efficiency ratio (FER) was calculated as the amount of intake per mouse divided by the weight of the mouse.

2.12. Hematoxylin and Eosin (H&E) Staining. Collected blood was allowed to separate for 2 h and centrifuged at 3,000 rpm for 15 min. Serum was collected and analyzed using a blood analyzer for triglyceride, glucose, total cholesterol, LDL, HDL, ALT, and AST levels.

2.13. Statistical Analysis. Statistical significance was analyzed using Graphpad Prism version 7.00 (San Diego, CA, USA) and one-way ANOVA with Dunnett’s posttest. P < 0.05 was considered significant. Data were presented as mean ± SD.

3. Results

3.1. UPLC-QToF MS Analysis and GCMS Analysis of U. parvifolia. Using UPLC-QToF MS, the compounds identified in ethanol extract of the bark of UP are as shown in Figure 1(a). Peak 1 was identified as +(-) catechin as compared to its standard shown in Figure 1(b), and peak 2 was identified as catechin-7-O-β-D-apiofuranoside as compared to its standard, as shown in Figure 1(c). The concentration of catechin detected is 6.14 mg/g, whereas that of catechin-7-O-β-D-apiofuranoside was 156.3 mg/g, as previously described [14]. GCMS analysis revealed that the main components are hexadecanoic acid and β-sitosterol (Table 2).

3.2. U. parvifolia Inhibited Production of Lipid in 3T3-L1 Preadipocytes. Lipid is produced when preadipocytes differentiate into adipocytes. UP reduced lipid production in differentiated 3T3-L1 preadipocytes (Figure 2(a)). The concentrations of UP used were not toxic, as determined by an MTT assay on 3T3-L1 cells (Figure 2(b)). Expression of adipogenic genes FAS, PPARγ, aP2/FABP4, C/EBPa, adipsin, IGF-1, ACC, adiponectin, leptin, AMPKα1, and SREBP1c/ADD1 was decreased with increasing concentrations of UP treatment (Figure 2(c)). However, AMPKα2 expression was increased. AMPK is a main regulator of metabolism, and its phosphorylated form was increased with increasing concentrations of UP treatment, while p-ACC protein expression was reduced (Figure 2(d)). No change was observed in ACC expression. The relative expressions of AMPKα and ACC were quantified using ImageJ software (NIH, USA), as shown in Figures 2(e) and 2(f).

3.3. U. parvifolia Inhibits Development of Obesity in Mice Fed an HFD. There were no significant differences in food and water intake between the groups (Figures 3(a)–3(c)). However, mice that were administered orlistat daily and UP 100 or UP 300 exhibited notable decreases in weight (Figure 3(e)). Orlistat group mice exhibited a significant reduction as compared to mice consuming only an HFD from week 5, whereas UP 100- and 300-treated groups exhibited a significant reduction from week 6. FER was significantly reduced in both UP-treated groups in a dose-dependent manner (Figure 3(d)). Subcutaneous adipose...
tissues were significantly reduced in the orlistat and UP 300-treated groups, whereas epididymal adipose tissue was reduced significantly only in UP 300 (Figure 3(f)). The weight of the liver has decreased in treated groups, whereas there was no significant change in kidney and spleen weight in all groups (Figure 3(g)). From the results, it can be seen that UP treatment significantly reversed weight gain in mice and reduced FER and weight of adipose tissue and the liver.

3.4. *U. parvifolia* Improved Histological Damage Induced by an HFD in Mice. There was a visible change in body size as shown in the representative pictures of mice for each group; mice were significantly increased in size in the HFD-group, whereas there was a visible reduction in size in mice from the groups treated with orlistat and UP 100 or UP 300 (Figure 4(a)). Liver tissue extracted after the mice were euthanized was compared (Figure 4(b)). There was a visible increase in size and change in coloration to a paler shade, indicating the occurrence of fatty liver after consuming an HFD. This change was improved with orlistat and UP 100 or UP 300 treatment. This observation was further confirmed with histological analysis of liver tissue and epididymal adipose tissue stained with H&E. The hepatocytes in HFD-treated mice were remarkably increased in size, and the pale coloration was visible due to accumulation of lipids. There was also a visible decrease in size of hepatocytes in the orlistat and UP-treated groups. Ballooning of hepatocytes, macrovesicular steatosis (indicated by arrows), and the foamy characteristics of the hepatocytes as depicted in microvesicular steatosis were also identified in the HFD-treated group, indicating the occurrence of nonalcoholic fatty liver disease (NAFLD). Infiltration of inflammatory cells was also observed (indicated by arrowheads) (Figure 4(c)). Therefore, treatment of orlistat and UP has remarkably inhibited the progression of NAFLD. The size of adipocytes in HFD-treated mice was significantly larger, as
Figure 2: Continued.
Figure 2: UP inhibits secretion of lipid droplets in 3T3-L1 preadipocytes. 3T3-L1 preadipocytes were differentiated or undifferentiated (UD) and treated with or without UP for 10 days before they were stained with oil red O (a). Cell viability was confirmed with an MTT assay after 3T3-L1 cells were treated with different concentrations of UP (b). Real-Time PCR was carried out by extracting RNA of differentiated 3T3-L1 cells with or without treatment of UP (c). Western blot analysis of p-AMPKα and p-ACC against the housekeeping gene β-Actin. 3T3-L1 cells were treated with or without UP after differentiation. After 10 days, protein was extracted from the cells, separated using SDS-PAGE, transferred to a PVDF membrane, incubated overnight with the primary antibody, incubated with secondary antibody, and developed (d). Expressions of genes were compared against the differentiated control group. Western blot was repeated in triplicate, and images were quantified using ImageJ (e and f). Statistical analysis was performed using one-way ANOVA with Dunnett’s posttest, and $P < 0.05$ was considered significant.

Figure 3: Continued.
compared to mice fed normal chow, indicating hypertrophy of adipocytes. Adipocytes were shown to decrease with treatment of orlistat and UP. UP 300 in particular reduced the size of adipocytes as compared to the adipocytes of mice fed normal chow. Adipocyte count per frame was also significantly recovered in UP 300-treated mice (Figure 4(d)). This has confirmed the efficacy of UP in reversing obesity induced with an HFD and demonstrates the therapeutic potential of this compound for increasing metabolism and reversing obesity.

3.5. *U. parvifolia* Increased Browning-Related Genes in Adipose WAT. Using real-time PCR, the expression of PGC-1α and UCP-1 were shown to increase in particular with treatment of UP 300 (Figures 5(c) and 5(e)). The products of real-time PCR were run on ethidium bromide-stained agarose gel for confirmation (Figure 5(a)). The protein expression of p-AMPKα was increased significantly, whereas the expression of p-ACC was decreased only in UP 300 (Figure 5(b)). No change was observed in ACC expression. The gel images were quantified using ImageJ (Figures 5(d) and 5(f)). Our results have shown that UP has induced browning of epididymal adipose tissue in mice fed an HFD.

3.6. Serum Biochemistry of Mice in a High-Fat Model. Serum triglyceride levels were significantly reduced by orlistat and UP 100 or UP 300 treatment. LDL levels were decreased with UP treatment. However, there were no significant decreases in glucose levels, total cholesterol, and HDL in any treated groups. ALT and AST are both markers of liver damage and did not show significant change in all groups (Figures 6(a)–6(g)).
Figure 4: Continued.
Figure 4: UP reduced the size of mice and mouse livers and improved the histology of the liver and adipose tissue induced by an HFD. Mice were fed with an HFD for a total of 12 weeks, and oral administration of orlistat and UP was carried out after 4 weeks of a daily HFD. Representative images of mice and livers for each group (a-b). Liver tissues and epididymal adipose tissue were dehydrated and fixed in paraffin and then sectioned before staining with H&E (c-d). Liver tissues (c) and epididymal adipose tissue (d) were observed at 100x. Arrows indicate balloon cells, and arrowheads indicate the infiltration of inflammatory cells. CV indicates the location of the central vein, whereas PV indicates the portal veins. Magnified image of the CV area of the liver tissue in HFD-treated group was taken at 200x. Statistical analysis for adipocyte count performed using one-way ANOVA with Dunnett’s posttest, and * indicates $P < 0.05$ compared to the HFD group, whereas # indicates a $P < 0.05$ as compared to the control group.

Figure 5: Continued.
Other than storing lipids, white adipose tissue also functions as an endocrine organ, secreting mainly adiponectin and leptin [30]. Additionally, white adipose tissues secrete TNF-α in an obese rodent model [31]. IL-6 was also detected in white adipose tissues in cases of obesity and insulin resistance [32]. Both TNF-α and IL-6 are also known as...
adipokines and proinflammatory cytokines. Secretion of adipokines by WAT is a response to hypoxia in areas of fat deposits in obese individuals, as the vasculature is insufficient to maintain normoxia throughout the WAT due to its constant expansion. This causes the adipocytes to be hypoxic, and an inflammatory response increases blood flow and stimulates angiogenesis [33]. Therefore, obesity should be treated not only merely as a metabolic disorder but also as the root cause of many chronic diseases.

3T3-L1 preadipocytes were used to investigate adipogenesis in vitro. Peroxisome proliferator-activated receptor y (PPARγ) and CCAAT/enhancer binding protein α (C/EBPα) are responsible for preadipocyte growth arrest and their differentiation into adipocytes. As the shift in gene expression indicates cellular differentiation, we studied the related gene expression for confirmation of preadipocyte differentiation. Our results have shown that PPARγ, C/EBPα, and SREBP1c/ADD1 are downregulated in 3T3-L1 preadipocytes after treatment with UP. Furthermore, leptin and adiponectin are also adipokines secreted by adipose tissue. Leptin in serum is elevated in the case of obesity as it functions to accelerate energy expenditure [34], whereas insulin levels are negatively correlated with adiponectin levels in 3T3-L1 preadipocytes [35]. Treatment with UP also reduced both leptin and adiponectin mRNA levels in 3T3-L1 preadipocytes in this study. Adipsin is upregulated in the presence of insulin in 3T3-L1 preadipocytes [36]. As FAS, ACC, and aP2/FABP4 are regulated by PPARγ [37,38], these genes were downregulated with UP treatment. Insulin growth factor 1 (IGF-1) is essential in the differentiation of 3T3-L1 preadipocytes [39], and its expression has been reduced with treatment of UP. AMPK is the master regulator of metabolism. Phosphorylation of AMPK inhibits the phosphorylation of ACC which then inhibits lipid synthesis and, at the same time, promotes fatty acid oxidation by increasing CPT1 (carnitine palmitoyltransferase 1) expression. As leptin inhibits the phosphorylation of AMPKa2 [40], suppression of leptin explains the increase of AMPKa2 (Figure 2(c)). UP has been shown to reduce lipid production and did not show signs of cytotoxicity in 3T3-L1 preadipocytes for the concentrations of UP used in vitro and additionally resulted in the downregulation of mRNA expressions of adipogenesis-related genes and increased expression of AMPKa2. Western blot analysis has also revealed that UP is capable of upregulating p-AMPKα and suppressing p-ACC (Figures 2(d)–2(f)).

PGC-1α was previously identified to be expressed only in BAT. Recent studies revealed the browning of WAT, also known as brite or inducible brown adipocytes [41]. Brown adipocytes are dominant in BAT, whereas beige adipocytes are found in WAT in cases of increased energy expenditure or exposure to cold. Therefore, the increase of beige adipocytes is marked by increases of PGC-1α and UCP-1 and results in increased energy expenditure that can then counter obesity. Brown adipocytes have large numbers of mitochondria and highly express UCP-1, which is located in the inner mitochondrial membrane. Fatty acids from triglycerides will undergo β-oxidation followed by conversion into chemical energy by mitochondrial UCP-1 [42]. Therefore, increasing the expression of PGC-1α and UCP-1 in white adipose tissue increases energy expenditure, which will reduce the amount of triglycerides stored in WAT. Our results have shown that mice fed with UP have markedly increased PGC-1α and UCP-1 expression in the epididymal adipose tissue of mice (Figure 5), indicating that UP increases the browning of WAT. The reduced weight of the mice and the respective adipose tissues in mice indicated the conversion of triglycerides in WAT to fatty acids which are then dissipated into heat energy through conversion by UCP-1, with no observed reduction of feed intake in all groups. UP also upregulated p-AMPKα expression and downregulated p-ACC expression in epididymal adipose tissue of mice fed a HFD, confirming the role of UP in countering obesity. These findings are further supported by evaluation of the levels of serum biomarkers, namely triglyceride, glucose, cholesterol, ALT, and AST, accompanied by the improvement in histological analysis of liver tissue and epididymal adipose tissue (Figures 4 and 6). UP increased lipid metabolism as there were lesser fat droplets deposited in the liver as observed in H&E staining, preventing NAFLD as previously reported [43].

UPLC-QToF MS was carried to identify the polar compounds in UP. Our findings revealed that the most abundant polar compounds in UP are catechin and catechin-7-O-β-D-apiofuranoside (Figure 1). Catechin has been known for antiobesity properties, explaining its possible contribution in the efficacy of UP in curbing obesity [44]. Catechin-7-O-β-D-apiofuranoside prevents hepatic fibrogenesis by inhibitng stellate cell activation [45]. GCMS analysis was carried out to identify the nonpolar constituents of UP. Our findings have shown that the major nonpolar compounds
are hexadecanoic acid and β-sitosterol (Table 2). While hexadecanoic acid has been reported to have anti-inflammatory properties [46], β-sitosterol has been reported to induce apoptosis of cancer cells, have hypocholesterolemic and antidiabetic properties, and increase the activity of NK cells [47]. Moreover, sitosterol has also been reported to reduce choric inflammation induced by obesity [48]. Taken altogether, we believe that β-sitosterol and catechin in UP are the main ingredients that induce WAT browning.

5. Conclusions

Our findings reveal that UP increased browning in WAT in mice, possibly acting through the PGC-1α/SIRT1/UCP-1 axis. Further studies should be conducted to confirm the role of SIRT1 and PRDM16. In conclusion, we have revealed the mechanism of action of UP on white adipocytes is via increased mitochondrial biogenesis in WAT, inducing an increase in energy expenditure and thermogenesis (Figure 7).

**Abbreviations**

WAT: White adipose tissue  
BAT: Brown adipose tissue  
UCP-1: Uncoupling protein 1  
PGC-1α: Peroxisome proliferator-activated receptor  
LDL: Low-density lipoprotein  
HDL: High-density lipoprotein  
ALT: Alanine aminotransferase  
AST: Aspartate aminotransferase.

**Data Availability**

The data used to support the findings of this study are included within the article.

**Conflicts of Interest**

All authors declare no conflicts of interest.

**Authors’ Contributions**

Y.Y.L. designed the research plan, conducted the research, analyzed data, and wrote the paper. M.K. and S-H.K. designed the research plan and conducted the research. I.M. partially conducted the research. D.-S.K., H.J.Y., S.E.L., S.K., and S-D.K. analyzed the data. M. H. R. has primary responsibility for the final content. All authors have read and approved the final manuscript.

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**References**


