Anti-obesity Effect of *Dioscorea oppositifolia* Extract in High-Fat Diet-Induced Obese Mice and Its Chemical Characterization

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*Dioscorea oppositifolia* is a well-known edible and traditional medicine for the treatment of gastrointestinal diseases. In our previous study, *D. oppositifolia* exhibited both pancreatic lipase inhibition and an anti-adipogenesis effect in vitro. This study was performed to investigate the anti-obesity effect of *D. oppositifolia* on high-fat diet-induced obese mice. Female ICR mice were fed a high-fat diet with the 100 mg/kg of *D. oppositifolia* n-BuOH extract for 8 weeks. The high-fat diet mice received the 15 mg/kg Orlistat orally as a positive control. The body weight, parametrial adipose tissue weight, and the levels of triglyceride (TG), total cholesterol (TC), and low density lipoprotein (LDL)-cholesterol in blood serum of female ICR were significantly decreased by feeding a high-fat diet with the n-BuOH extract of *D. oppositifolia*. An inhibitory effect of *D. oppositifolia* extract on dietary fat absorption was also clearly shown. The *D. oppositifolia* sample was found to contain 3,5-dimethoxy-2,7-dihydroxyphenyl)-3,5-heptanediol as main components based on its phytochemical analysis. The present study is the first report of the anti-obesity effect by *D. oppositifolia* n-BuOH extract using an established disease model. The increase in fecal fat excretion by treatment of *D. oppositifolia* may be an effective approach for treating obesity and related diseases.

**Key words** *Dioscorea oppositifolia*; anti-obesity; high-fat diet-induced obese mouse; fecal fat excretion; phytochemical analysis

Obesity is the disease associated with accumulation of excessive body fat resulting from energy imbalance. The obesity epidemic has become a public health problem, leading to secondary chronic diseases like dyslipidemia, cardiovascular disease, and type 2 diabetes. Furthermore, obese persons experience a number of physical problems such as sleep apnoea and joint pain, which shortens the life expectancy and impacts on the quality of life. Natural phytochemicals have become more attractive as sources of industrial and medicinal materials in overweight and obese patients due to potential anti-obesity properties. Numerous trials have been conducted to discover pharmacological features of crude plant extracts, isolates, and phytochemical combinations against obesity. Nevertheless, attention has continuously focused on new anti-obesity agents from herbal resources to minimize adverse effects associated with the present anti-obesity drugs.

*Dioscorea oppositifolia* L. (Dioscoreaceae, synonym: *D. opposita Thunb.*) has been cultivated in China, Japan, and Korea as a food and widely used as a traditional medicine for a long time. Our current pharmacological studies showed that *D. oppositifolia* possesses significant inhibitory activity against porcine pancreatic lipase in vitro. In addition, the anti-adipogenesis effect of *D. oppositifolia* n-BuOH soluble extract has been detected in cultured 3T3-L1 adipocyte. Biological activities of *Dioscorea* species against diverse metabolic disorders including diabetes and obesity have been already documented. However, anti-obesity effect of certain extract derived from *D. oppositifolia* in animal model remains to be defined. Here, based on the previous in vitro results of *D. oppositifolia*, we investigated the direct effect of *D. oppositifolia* n-BuOH extract on obesity induced by high-fat diet in animal model. Changes in body weight and serum, hepatic, and fecal lipid parameters were measured to identify the potential therapeutic effect of *D. oppositifolia* extract on an experimental model of obesity.

**MATERIALS AND METHODS**

**Plant Material and Sample Preparation** The rhizomes of *D. oppositifolia Thunb.* (Dioscoreaceae) were provided by Tong Yang Moulsan Co., Ltd. (Nonsan, South Korea), and identified by Prof. Gwang Jin Chang of the Korea National College of Agricultural and Fisheries. A voucher specimen (SNUPH-0822) has been deposited in the Medicinal Herb Garden, Seoul National University. Fresh rhizomes of *D. oppositifolia* (19 kg) were sliced into small pieces and lyophilized. The dried samples were extracted with 95% EtOH (20 L×3) and evaporated under reduced pressure. The 95% EtOH extract (380 g) was suspended in distilled water (600 mL) and the suspension was partitioned with CHCl₃ (1 L), EtOAc (1 L) and n-BuOH (1 L), sequentially. Previous in vitro study showed that the n-BuOH extract of *D. oppositifolia* and its constituents possess pancreatic lipase inhibitory activity. We carried out following animal experiment to determine the anti-obesity property of the *D. oppositifolia* n-BuOH extract (DOB). In addition, in order to discover the composition of DOB, phytochemical analysis has been done using an Agilent HPLC-TOF/MS system (Agilent Technologies, U.S.A.).

**Animals and Diets** Female ICR mice (3 weeks old) were obtained from the Experimental Animal Breeding Center of Seoul National University (Seoul, Korea). Animals were acclimatized for one week under a 12:12 h light–dark cycle.
and constant temperature (20±2°C) and humidity (50±5%), with water and food freely available. The mice were divided into 4 groups and then fed with the normal diet (10% kcal fat, 3.85 kcal/g) and high-fat diet containing 60% kcal fat (5.24 kcal/g) (Research Diets Inc.; New Brunswick, NJ, U.S.A.), respectively. One hundred milligrams per kilogram body weight of DOB dissolved in 0.5% carboxymethyl cellulose (CMC) was orally administered to sample treatment group once a day for 8 weeks. The high-fat diet mice received either 15 mg/kg body weight Orlistat (Zenical®, Roche Pharm Ltd., Reinach, Switzerland) or 0.5% CMC (10 mL/kg body weight) orally for 8 weeks as a positive or negative control. A normal group fed with laboratory pellet chow was also treated with only 0.5% CMC (10 mL/kg body weight) as a vehicle. All of the animal experiments were carried out according to the guidelines of the Seoul National University’s Committee on the Care and Use of Laboratory Animals (SNU-110412-6).

Measurement of Body, Liver, Adipose Tissue Weight, and Food Intake  The body weight and food intake were measured under the condition of non-fasting once and twice a week, respectively. The food intake efficiency was evaluated by monitoring the food consumption (g) in each cage for the experimental period, and was calculated on per animal per day basis. After 8 weeks of normal or high-fat diet feeding, mice were sacrificed by cervical dislocation. Blood samples were obtained from the abdominal aorta and centrifuged at 1500rpm for 15 min to separate to plasma and blood cells.12) The liver and parametrial adipose were dissected, weighed and stored at −80°C until analysis.13)

Biochemical Analysis of Plasma Samples  The plasma levels of triglyceride (TG), total cholesterol (TC) and high-density lipoprotein cholesterol (HDLc) were estimated using assay kits (Asan Pharmaceutical Co.; Seoul, Korea). Low-density lipoprotein cholesterol (LDLc) was measured using the Friedewald’s formula: 

\[ \text{LDLc} = \text{TC} - \text{HDLc} - \text{TC} \times \text{TG} \]

The atherogenic index (AI) was calculated according to the method of Aziz et al.: 

\[ \text{AI} = (\text{TC} - \text{HDLc}) / \text{HDLc} \]

Plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentrations were determined spectrophotometrically using the glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) assay reagents from Asan Pharmaceutical Co.

Determination of Hepatic and Fecal Lipid Content  Hepatic and fecal lipids were extracted according to previous protocol with slight modification.16) Liver tissue (200 mg) or freeze-dried feces (0.5 g) were homogenized in 1 mL of distilled water and 5 mL of methanol–chloroform solution (1:1, v/v). The mixture was extracted by shaking horizontally for 10 min and centrifuged at 2000×g for 10 min. The lower phase was dried and total lipid was measured. TG and TC contents in feces were determined using the assay kits.

Phytochemical Analysis by HPLC-Time of Flight (TOF)-MS  The n-BuOH fraction of D. oppositifolia rhizomes was analyzed using an Agilent 6530 Accurate-Mass Q-TOF LC/MS system (Agilent Technologies) for phytochemical characterization. A Poroshell 120 EC-C18 column (3.0×100mm, 2.7 μm, Agilent) was used for analysis at a flow rate of 0.3 mL/min. The mobile phase consisted of acetonitrile (solvent A) and water (solvent B), using a linear gradient elution: 5–95% A (0–20 min); 10% A (20–30 min). All acquisitions were performed under positive ionization mode. Mass spectra were recorded across the range m/z=100–1500 with accurate mass measurement of all mass peaks.

Statistical Analysis  Each data value was presented as the mean±standard deviation (S.D.) Statistical analysis body weight change in vivo experiment analyzed by two-way ANOVA with between groups and days. The data were considered to be significant statistically if the probability had a value of 0.05 or less.

RESULTS

Anti-obesity Effect of D. oppositifolia Extract on Body Weight Gain, Liver, Adipose Tissue Weight and Food Intake in Mice Fed a High-Fat Diet  The body weight changes of the mice fed a normal diet or high-fat diet or Orlistat or DOB were estimated during the treatment period once a week. After 8 weeks of treatment, the average body weight of high-fat diet-fed mice was considerably higher than that of the other groups. The body weight in mice fed a high-fat diet alone was 40.07±3.55 g, which was approximately 32.6% higher than that of the normal diet group (30.21±1.11 g). The consumption of DOB and positive control Orlistat (a potent pancreatic lipase inhibitor) significantly decreased the gain in body weight. The treatment of DOB and Orlistat prevented increase in weight gain by 45.2% (p<0.01) and 47.3% (p<0.001), respectively (Fig. 1 and Table 1). Treatment with DOB markedly reduced cumulative parametral fat and liver weight relative to non-treated mice (Table 1). The parametral adipocyte tissue weight (2.7-fold of control) and liver weight (1.3-fold of control) were elevated in animals fed a high-fat diet when compared to the normal diet group. Increased parametral adipose tissue in high-fat diet-fed mice was prevented by treatment of DOB to 22.5% (p<0.01). Furthermore, DOB prevented liver weight gain by 30.0% (p<0.05) compared with their respective control.

Effect of D. oppositifolia Extract on Serum Lipid Contents in Mice Fed a High-Fat Diet  Serum concentrations of TG, TC, and HDLc of the four groups of mice are shown in Table 2. A significant increase in the levels of TG and TC was induced by feeding high-fat diet. Conversely, the plasma HDLc concentration in the high-fat diet-fed mice was less than that of the normal diet-fed mice. DOB or Orlistat treatment markedly prevented the elevation of serum TG and TC by 18.8 and 15.4%, respectively, compared to untreated group. Moreover, the DOB treatment increased the level of HDLc to 34.2%, leading to TC/HDLc ratio improvement. The ratio of TC to HDLc is considered a better indicator of coronary heart disease risk than individual lipoprotein concentration.17) The value of TC/HDLc in the high-fat diet group was 3.55, whereas the ratio in mice treated with DOB (1.80) was much lower.

D. oppositifolia Extract Prevented Lipid Accumulation in Mice Liver Fed a High-Fat Diet  Feeding a high-fat diet to mice for 8 weeks developed fatty liver (Table 2). The hepatic TG and TC concentrations of the high-fat diet group were dramatically increased by 3.0-fold and 2.85-fold, respectively, compared to the normal diet group. DOB and Orlistat treated mice, however, prevented increase in hepatic TG content by 48.1 and 58.6%, respectively, in comparison with the high-fat diet group. The hepatic TC concentration was also reduced by DOB (46.9% decrease) and Orlistat (57.1% decrease) treatments. The effect of DOB on liver function was evaluated
TG, triglyceride; TC, total cholesterol; HDLC, high-density lipoprotein cholesterol; LDLC, low-density lipoprotein cholesterol; AI, atherogenic index. LDLC and AI were estimated indirectly by using formulas: LDLC = TC – HDLC – TG/5; AI = (TC – HDLC)/HDLC. AST, aspartate aminotransferase; ALT, alanine aminotransferase.

Table 1. Changes in Body Weight, Liver, Adipose Tissue Weight, and Food Intake Efficiency of High-Fat Diet-Induced Obese Mice Treated with DOB

<table>
<thead>
<tr>
<th></th>
<th>Normal diet</th>
<th>High-fat diet</th>
<th>D. oppositifolia extract</th>
<th>Orlistat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final body weight (g)</td>
<td>30.21±1.11</td>
<td>40.07±3.55</td>
<td>32.70±2.02</td>
<td>32.07±2.65</td>
</tr>
<tr>
<td>Liver weight (g/100 g body weight)</td>
<td>5.06±1.51</td>
<td>6.56±0.49</td>
<td>4.59±1.55</td>
<td>4.91±0.84</td>
</tr>
<tr>
<td>Parametrial adipose tissue weight (g/100 g body weight)</td>
<td>3.29±0.58</td>
<td>8.93±1.67</td>
<td>6.92±1.86</td>
<td>5.40±1.55</td>
</tr>
<tr>
<td>Body weight gain (g/8 weeks)</td>
<td>6.71±1.11</td>
<td>15.97±3.55</td>
<td>8.75±2.65</td>
<td>8.42±1.65</td>
</tr>
<tr>
<td>Food intake (g/8 weeks)</td>
<td>1350±14.28</td>
<td>1420±10.62</td>
<td>1308±17.00</td>
<td>1218±11.56</td>
</tr>
<tr>
<td>Food intake efficiency</td>
<td>0.009±0.001</td>
<td>0.015±0.003</td>
<td>0.009±0.003</td>
<td>0.009±0.002</td>
</tr>
</tbody>
</table>

Values are expressed as the mean±S.D. (n=7). a) p<0.01, b) p<0.001, compared with the normal diet group; c) p<0.05, d) p<0.01, e) p<0.001, compared with the high-fat diet group. D. oppositifolia extract group is high-fat diet mice treated with 100mg/kg body weight/d D. oppositifolia n-BuOH extract; Orlistat group is high-fat diet mice treated with 15mg/kg body weight/d Orlistat. Food intake efficiencies were calculated by body weight gain per calories consumed.

Table 2. Serum and Hepatic Lipid Profiles of Mice Treated with DOB

<table>
<thead>
<tr>
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<th>High-fat diet</th>
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<th>Orlistat</th>
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</thead>
<tbody>
<tr>
<td>Serum parameters</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>TG (mg/100 mL)</td>
<td>87.41±12.49</td>
<td>147.47±23.86</td>
<td>119.70±11.84</td>
<td>94.71±11.23</td>
</tr>
<tr>
<td>TC (mg/100 mL)</td>
<td>86.52±18.15</td>
<td>130.74±8.24</td>
<td>110.62±6.62</td>
<td>100.05±13.90</td>
</tr>
<tr>
<td>HDLC (mg/100 mL)</td>
<td>39.31±1.57</td>
<td>30.32±7.70</td>
<td>40.69±5.02</td>
<td>41.21±2.59</td>
</tr>
<tr>
<td>LDLC (mg/100 mL)</td>
<td>38.99±6.65</td>
<td>77.67±10.27</td>
<td>50.68±10.50</td>
<td>49.33±14.88</td>
</tr>
<tr>
<td>AI</td>
<td>1.20±0.34</td>
<td>3.55±1.05</td>
<td>1.80±0.35</td>
<td>1.44±0.32</td>
</tr>
<tr>
<td>Hepatic pathology</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Total lipid (mg/g liver)</td>
<td>41.45±1.14</td>
<td>87.45±6.23</td>
<td>62.75±2.13</td>
<td>42.38±0.84</td>
</tr>
<tr>
<td>TG (mg/g liver)</td>
<td>14.05±1.22</td>
<td>42.08±13.76</td>
<td>21.84±2.44</td>
<td>17.41±6.57</td>
</tr>
<tr>
<td>TC (mg/g liver)</td>
<td>13.80±1.11</td>
<td>39.29±12.51</td>
<td>20.88±2.22</td>
<td>16.85±5.97</td>
</tr>
<tr>
<td>Plasma AST</td>
<td>26.32±7.31</td>
<td>89.82±9.52</td>
<td>59.17±8.24</td>
<td>120.43±11.01</td>
</tr>
<tr>
<td>Plasma ALT</td>
<td>40.11±3.93</td>
<td>53.35±7.19</td>
<td>40.12±3.68</td>
<td>48.00±9.23</td>
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**The Inhibition of Fat Absorption by D. oppositifolia Extract in Mice Fed a High-Fat Diet**

Orlistat is a potent pan-pancreatic lipase inhibitor, thereby reducing TG absorption and fatty acid accumulation in intestine. By measuring the serum aminotransferase activities. Plasma level of ALT was slightly increased in high-fat diet group (53.35 ± 7.19) compared to normal diet control (48.00 ± 9.23). The activity of ALT was regressed by 24.8% in DOB-treated animals. AST was distinctly decreased by treatment of DOB as well (34.1% decrease), suggesting that DOB might have a beneficial effect on the liver damage induced by high-fat diet.

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DISCUSSION

Avoidance of obesity or weight reduction in overweight individuals is likely to prevent increases in the metabolic complications including diabetes, hypertension, dyslipidemia, and some cancers.\(^{19,20}\) The prevalence of comorbidities related to obesity and overweight emphasizes that concerted and sustained efforts should be made to prevent and treat obesity rather than just its associated comorbidities.\(^{25}\)

The present study was performed to determine the \textit{in vivo} anti-obesity effect of \textit{D. oppositifolia} n-BuOH fraction (DOB) in animal model based on its \textit{in vitro} inhibitory activity against pancreatic lipase.\(^{8}\) When obese mice (high-fat diet fed) were treated with DOB for 8 weeks, body weight and adipose tissue growth were apparently reduced as compared with the high-fat diet untreated group. In particular, the food intake efficiency of DOB group was lower than that of high-fat diet group. The ratio of body weight gain to cumulative food calories is described as the food intake efficiency. Previous researches indicated that feeding a high-fat diet to mice caused a decrease in energy expenditure and an increase in food intake efficiency.\(^{20,25}\) The mice fed a high-fat diet increased the food intake efficiency up to 1.7-fold of normal diet group in this study. In contrast, the feeding efficiency of obese mice treated with DOB was similar to that of normal diet group. As a result, DOB-treated mice are considered to obtain less energy from the high-fat diet compared with their counterparts.

Administration of high-fat diet caused changes in parameters of hepatotoxicity (AST and ALT) as well as lipid metabolism (HDLC and TG) in animal model. The activities of AST and ALT were significantly enhanced in high-fat diet-fed mice, suggesting a hepatic toxicity tendency. Two representative anti-obesity drugs, Orlistat and Sibutramine, have been reported to increase in both AST and ALT levels in high fat diet-induced animal even if they have beneficial effects toward suppressing obesity.\(^{24,25}\) Our result also showed the up-regulation of AST in Orlistat-treated mice in accordance with previous literatures. In comparison, serum AST and ALT levels, the hepatotoxicity markers, were significantly decreased in \textit{D. oppositifolia}-treated high-fat diet-fed mice at end of the study. This might be an indication that \textit{D. oppositifolia} has no adverse influence on the hepatic enzymes, therefore appears to be a safe and effective agent when used to manage obesity.

As a principle lipolytic enzyme involved in the hydrolysis of dietary fat, pancreatic lipase serves as an interesting target in the development of anti-obesity agents.\(^{29}\) Natural sources have proven to be potential inhibitors for pancreatic lipase, which is closely involved in the process of lipid absorption and anti-obesity therapeutics.\(^{27}\) Plant materials like \textit{Cassia mimosoides} (ethanol extract) and \textit{Salacia reticulate} (water extract), which act as pancreatic lipase inhibitors, have been reported to suppress body weight gain in animal model.\(^{28,29}\) Our previous data exhibited that the DOB down-regulates porcine pancreatic lipase activity to 57% at a concentration of 10 µg/mL \textit{in vitro}.\(^{8}\) In the present study, high-fat diet-induced obese mice treated with DOB (100 mg/kg body weight) elevated fecal lipid, TG, and TC output to 1.3-, 1.7-, and 1.4-fold of control, respectively. The increase in fecal fat excretion of the DOB-treated obese mice was correlated with its pancreatic lipase inhibitory activity as well as the prevention of increase in body weight. Taken together, \textit{D. oppositifolia} seems to inhibit body weight gain through the modulation of lipid absorption or lipid metabolism rather than reduction in food intake.

Fingerprint analysis and compound determination was performed using an established HPLC-TOF/MS method. The n-BuOH extract of \textit{D. oppositifolia} was found to contain 3,5-dimethoxy-2,7-phenanthenediol, batatasin I, (4E,6E)-7-(4-hydroxy-3-methoxyphenyl)-1-(4-hydroxyphenyl)-4,6-heptadien-3-one, batatasin V, and (3R,5R)-3,5-dihydroxy-1,7-bis(4-hydroxyphenyl)-3,5-heptanediol. Out of the components of DOB, 3,5-dimethoxyphenanthenre-2,7-diol and batatasin I were identified as non-polar standard maker compounds from \textit{D. oppositifolia} rithomes in the previous publications.\(^{18,30}\) The contents of two main compounds in DOB, 3,5-dimethoxyphenanthenre-2,7-diol and (3R,5R)-3,5-dihydroxy-1,7-bis(4-hydroxyphenyl)-3,5-heptanediol, were 10.42 and 21.23%, respectively.

Conclusively, \textit{D. oppositifolia} n-BuOH extract, including 3,5-dimethoxyphenanthenre-2,7-diol and (3R,5R)-3,5-dihydroxy-1,7-bis(4-hydroxyphenyl)-3,5-heptanediol as major components, efficiently reduced fat accumulation in high-fat diet-induced obese mice. The prevention of body weight gain by \textit{D. oppositifolia} extract was mediated through suppression of feeding efficiency and fat absorption. Further study is needed to confirm the anti-obesity effect of \textit{D. oppositifolia} in the obese human.

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Conflict of Interest The authors declare no conflict of interest.

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