Ethanol extract of lotus (Nelumbo nucifera) root exhibits an anti-adipogenic effect in human pre-adipocytes and anti-obesity and anti-oxidant effects in rats fed a high-fat diet

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\textbf{ABSTRACT}

Lotus (Nelumbo Nucifera) root, a well-known medicinal plant in Asia, is reported to have various therapeutic benefits, including anti-diabetes, anti-hypertension, and anti-hyperlipidaemia. We hypothesized that the ethanol extract of lotus root (ELR) would exhibit an anti-adipogenic effect in human pre-adipocytes as well as anti-obesity and anti-oxidant effects in rats fed a high-fat diet. Treatment with ELR in human pre-adipocytes resulted in inhibition of lipid accumulation and attenuated expression of adipogenic transcription factors such as peroxisome proliferator-activated receptor gamma and adipocyte marker genes, such as glucose transporter 4 and leptin. Administration of ELR resulted in a significant decrease in relative weights of adipose tissues in rats fed a high-fat diet. Consumption of a high-fat diet resulted in an increase in serum total cholesterol (TC) and triglyceride (TG) levels; however, administration of ELR resulted in a decrease in the levels of TC and TG. Administration of ELR resulted in a decrease in the level of serum leptin and insulin. Administration of ELR in rats fed a high-fat diet resulted in a decrease in hepatic thiobarbituric acid reactive substance content, elevated by a high-fat diet and an increase in superoxide dismutase activity and hepatic glutathione content. These results suggest that lotus root exerts anti-oxidant and anti-obesity effects and could be used as a functional and nutraceutical ingredient in combatting obesity-related diseases.

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\textit{Abbreviations:} ANOVA, analysis of variance; CE, catechin equivalent; DPPH, 2,2-diphenyl-1-picrylhydrazyl; EC\textsubscript{50}, efficient concentration of ELR decreasing initial DPPH concentration by 50%; E-fat, epididymal fat; ELR, ethanol extract of lotus root; GAE, gallic acid equivalent; GLUT4, glucose transporter; GSH, glutathione; HDL-C, High-density lipoprotein-cholesterol; IL, interleukin; LDL-C, low-density lipoprotein cholesterol; OD, optical density; PPAR-\gamma, peroxisome proliferator-activated receptor gamma; R-fat, retroperitoneal fat; RT-PCR, reverse transcription-polymerase chain reaction; SD, Sprague–Dawley; SEM, standard error of the mean; SOD, superoxide dismutase; SPSS, statistical product and service solutions; TBARS, thiobarbituric acid reactive substances; TC, total-cholesterol; TG, triglyceride.

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1. Introduction

Obesity is an international health problem caused by various environmental and genetic factors [1]. Excess energy accumulates in adipose tissue in the form of triglyceride. Many studies have reported that consumption of a high-fat diet induces abdominal obesity related to dyslipidaemia [2–4] and is related to the development of cancer [5]. This dyslipidaemia can lead to the development of various metabolic diseases, such as atherosclerosis, cardiovascular disease, and diabetes mellitus [6].

There are many different treatments to help control obesity, including diet, exercise, behavior modification, and medication [7–9]. Among these treatments, diet, exercise, and behavior modification are essential to the management of obesity; although often, their outcomes are not satisfactory. Various anti-obesity drugs, including orlistat, phentermine, mazindol, phendimetrazine, and diethylpropion have been approved by the Korea Food and Drug Administration. However, since these drugs have known side effects that include headaches, vomiting, and heart attacks, and orlistat is the only drug approved for long-term weight control [10], many researchers are interested in exploiting safe and effective natural products from various edible plants, such as fruits, vegetables, and herbs for the prevention or treatment of obesity.

Lotus (Nelumbo Nucifera) is a well-known medicinal plant in Asian countries, including Korea, Japan, and China. Extracts of leaf, root, seed, and flower are reported to have significant therapeutic benefits [11]. Among the various parts of lotus, the root is the most widely consumed. Fresh root contains abundant starch and minerals, including calcium, copper, iron, magnesium, and zinc [12]. The lotus root has many potential benefits, including antiinflammatory properties [13], antimicrobial activities [14], antifungal and anti-yeast activities [15], hypotension effect [16], hypolipidemic effect [17], hypoglycaemic activity [18], psychopharmacological activity [19], alleviation of hepatic steatosis [20], and diuretic activity [21]. Among various vegetables, the lotus root exhibited the strongest anti-oxidant activity [22]. However, most researchers have studied the in vitro anti-oxidant effect of lotus root, and in vivo studies are limited. In addition, although hyperlipidaemia is potentially related to obesity [2], studies of the anti-obesity effect of lotus root are not adequate.

Obesity induces oxidative stress, which may be a cause of obesity-related chronic diseases, such as diabetes, hypertension, and cardiovascular diseases [23–25]. If lotus root’s anti-oxidant effect has an anti-obesity effect, lotus root could be effective in the prevention and therapies for obesity-related chronic diseases.

Therefore, the objective of this study was to test the hypothesis that ELR would exhibit an anti-adipogenic effect in human pre-adipocytes and anti-oxidant and anti-obesity effects in rats fed a high-fat diet. In order to test this hypothesis, we first induced differentiation of human pre-adipocytes and measured lipid accumulation by oil red O staining, in order to determine the anti-adipogenic effect. Second, to determine the anti-obesity effect, we adminis-tered ELR to rats fed a high-fat diet and measured body weight gain, adipose tissue weight, serum lipid level, serum insulin, and adipokine levels. Finally, to determine the anti-oxidant effect, we measured the DPPH radical scavenging activity of ELR and hepatic thiobarbituric acid reactive substance (TBARS) content, hepatic glutathione (GSH) content, and serum superoxide dismutase (SOD) activity in rats fed a high-fat diet and administered ELR.

2. Methods and Materials

2.1. Preparation of lotus root and determination of total phenolic and flavonoid content of ELR

Raw lotus root was purchased from a lotus farm in Siheung (Kyunggi-do, Korea). The root was frozen in liquid nitrogen and stored at −20°C until extraction. The frozen lotus root (6.5 kg) was ground and extracted using 32.5 L of 95% ethanol at 50°C in a water bath for 2 hr. After vacuum filtration, the extract, which was evaporated using a rotary evaporator under reduced pressure, left a dry extract (208.8 g). The powder was obtained with an extraction yield of 3.2% and stored in desiccators under reduced pressure until application.

Analysis of total phenolic contents was performed using the Folin-Ciocalteu assay [26], and total flavonoid contents were determined using the colorimetric method [27], as described previously [28].

2.2. DPPH radical scavenging activity

Analysis of the free radical scavenging activity of ELR was performed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method modified from the method of Kuo et al. [29], and the results were compared with those for free radical scavenging activity of ascorbic acid. The methanol solution of DPPH (100 μM, 5 mL) was reacted with different concentrations of various ELR samples (0–20 μg/mL, 0.6 mL) and then kept for 30 min. The absorbance was measured on a Cary 300 UV–vis spectrophotometer (Agilent Technologies, Santa Clara, CA, USA) at 517 nm. The efficient concentration of ELR causing a decrease in initial DPPH concentration by 50% (EC50) was calculated.

2.3. Cell culture, Oil red O staining, and reverse transcription-polymerase chain reaction (RT-PCR)

Human pre-adipocytes were purchased from Cell Applications (San Diego, CA, USA) and maintained using a pre-adipocyte growth medium kit (Cell Applications, San Diego, CA, USA). Pre-adipocytes were seeded into six-well plates (1.8 × 10^5 cells/well in 2 ml of medium) and cultured until confluent. For differentiation, the culture medium was changed to adipocyte differentiation medium (Cell Applications, San Diego, CA, USA) and then cultured for two weeks by changing the medium every three days in the presence or absence of ELR at different concentrations (0, 25, 50, 100, and 200 μg/mL).

Lipid accumulation was examined with Oil red O staining, as described previously [28]. Microscopic images (Olympus, Tokyo,
Table 1 – Sequences for primers set by RT-PCR

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Forward Primer Sequences</th>
<th>Backward Primer Sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPAR-γ</td>
<td>TCGGTTTCAGAATGCGCTTG</td>
<td>AGTCAGCGGACCTCTGGATT</td>
</tr>
<tr>
<td>Leptin</td>
<td>GCGAGTCAGTCTCCTCCCAA</td>
<td>GTTCTCCAGGTCGTTGGAT</td>
</tr>
<tr>
<td>GLUT4</td>
<td>TGGCTGACCTGAAAGGATGAG</td>
<td>CCAACAAACGGGAGGACAAG</td>
</tr>
<tr>
<td>β-actin</td>
<td>TCATAGGATTAGCATCAGGG</td>
<td>CTTTCACAATGAGCTGGTG</td>
</tr>
</tbody>
</table>

PPAR-γ, peroxisome proliferator-activated receptor gamma. GLUT4, glucose transporter 4

2.5. Sampling and tissue preparation

At the end of the experimental period (after 14 weeks), following 12 hours of fasting, the animals were anesthetized with ether. Blood was collected from the heart and serum was obtained by centrifugation at 1650 × g for 20 minutes. The liver, kidney, spleen, epididymal fat (E-fat), and retroperitoneal fat (R-fat) were weighed. Some liver and E-fat was removed from the rats for histological photographs.

For tissue preparation, approximately 3 grams of minced liver tissue was homogenized with 6 ml cold potassium phosphate buffer (154 mM KCl, 50 mM Tris-HCl, 1 mM EDTA buffer, pH 7.4), as described previously [20]. The homogenate was centrifuged for 10 min at 1000 × g for removal of the precipitate, then 1.2 ml of the above mentioned supernatant solution was stored for analysis of thiobarbituric acid reactive substances (TBARS) and glutathione (GSH). The remaining supernatant solution was centrifuged for 20 min at 100,000 × g for removal of the microsome. Cytosol was obtained by centrifuging the supernatant solution for 60 min at 100,000 × g for removal of the microsome, using an ultracentrifuge (Centrifuge T-2080, Kontron Ins. Swiss). All procedures were performed at 0–4°C. The serum and all of the samples were immediately frozen in liquid nitrogen, and then stored in a −70°C deep freezer (Operon, Kimpo, Korea) until analysis.

Table 2 – Composition of experimental diets

<table>
<thead>
<tr>
<th>Component</th>
<th>Normal diet (g)</th>
<th>High fat diet (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredients (g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Casein</td>
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<td>200.0</td>
</tr>
<tr>
<td>Corn starch</td>
<td>447.486</td>
<td>155.036</td>
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<tr>
<td>Sucrose</td>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
<td>Dextrose</td>
<td>132.0</td>
<td>132.0</td>
</tr>
<tr>
<td>Cellulose</td>
<td>50.0</td>
<td>50.0</td>
</tr>
<tr>
<td>AIN-93 Vitamin</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>AIN-93 Mineral</td>
<td>35.0</td>
<td>35.0</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>70.0</td>
<td>25.0</td>
</tr>
<tr>
<td>Lard</td>
<td>-</td>
<td>175.0</td>
</tr>
<tr>
<td>L-cystine</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Choline Bitartrate</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>TBHQ</td>
<td>0.014</td>
<td>0.014</td>
</tr>
<tr>
<td>Energy (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein</td>
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<td>20</td>
</tr>
<tr>
<td>Lipid</td>
<td>16</td>
<td>45</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>64</td>
<td>35</td>
</tr>
</tbody>
</table>

TBHQ: tert-butylhydroquinone

Japan) of the stained cells were obtained after removal of the staining solution. The dye retained in the cells was eluted with isopropanol and optical density (OD) values were measured at the optical absorbance of 500 nm, using an Emax microplate reader (Molecular Devices, Sunnyvale, CA, USA). For assessment of cell viability, the cell proliferation assay was performed using a cell counting kit-8 (Dojindo Lab., Tokyo, Japan), as described previously [28].

Total RNA was isolated from human adipocytes using trizol (Invitrogen, Carlsbad, CA, USA). Complementary DNA synthesis was performed using 1 μg total RNA in a 20 μl reverse transcription reaction mixture containing 5 mmol/L MgCl₂, 10× RT buffer, 1 mmol/L dNTP, 1 U/μL RNase inhibitor, 0.25 U/μL AMV reverse transcriptase, and 2.5 μmol/L random 9-mers. The PCR conditions for leptin, glucose transporter 4 (GLUT4), and β-actin have been described previously [28]. The RT-PCR products were electrophoresed in 1.5% agarose gels containing ethidium bromide, and the bands were visualized under a Bio-Rad universal hood II (Bio-Rad Laboratories, Segrate, Italy). The primers were synthesized by Bioneer Co. Ltd (Seoul, Korea) (Table 1).

2.4. Animals and diet

Male Sprague-Dawley (SD) rats (four-week-old) were purchased from Koatech (Anseong, Korea). In accordance with the guidelines outlined by the Experimental Animal Ethics Committee, all rats were kept in laboratory animal housing at Kyung Hee University. All rats were kept under a constant 12 h light and dark cycle (AM 07:00-PM 07:00), with controlled temperature (22 ± 1°C) and humidity (55 ± 10%), following one week of acclimatization with a pelletized commercial diet, rats were randomly divided into two groups (normal (N) and high fat diet group). After the first seven weeks, in order to minimize error, rats in the high fat group were re-randomized into two sub-groups (high fat diet, HF group; high fat diet + ELR, HFR group).

The composition of the experimental diet was based on AIN-93 [30], as shown in Table 2. According to the Korean Nutrition Society and WHO [31], daily recommendation of vegetables is 350–400 g. In order to extrapolate doses of ELR from human to rat, we used a formula for dose translation based on body surface area [32]. ELR (1,200 mg/kg/day) was administered by oral gavage once per day to HFR and the same amount of saline solution was administered orally to the N and HF groups. Food and water intake was measured twice per week, and body weight was measured once per week.
2.6. Biochemical analysis.

Analyses of serum triglyceride (TG) and total-cholesterol (TC) levels were performed using an automatic analyser (BPC BioSed srl, Rome, Italy). High-density lipoprotein-cholesterol (HDL-C) was obtained from the whole serum using a high density lipoprotein precipitation reagent (Asan Pharm CO., Gyeonggi, Korea) after precipitation of low-density lipoprotein and very-low-density lipoprotein for 10 minutes at 800 x g, and then followed by analysis for HDL-C using the same method that was used for TC. Assayed chemistry control (Bio-Rad Laboratories, Irvine, CA, USA) was used for calibration, and all results were expressed as mg/dl. Serum low-density lipoprotein cholesterol (LDL-C) values were calculated using the Friedewald formula [33] as follows:

\[ \text{LDL - C} = \text{TC} - (\text{HDL - C} + \text{TG}/5) \]

Analysis of serum adiponectin level was performed using the rat single plex adiponectin 96 Well Plate MILLIPLEX map kit (Millipore Corporation, Billerica, MA, USA), and serum insulin and leptin levels were determined using the rat serum adipokine 96 Well Plate MILLIPLEX map kit (Millipore Corporation, Billerica, MA, USA) through luminex 100 (MiraiBio, Inc., Alameda, CA, USA).

Analysis of hepatic lipid peroxide content was performed using the thiobarbituric acid (TBA) method as described by Ohkawa et al. [34]. In brief, liver homogenate was added to a falcon tube containing 1% phosphoric acid and TBA reagent. After mixing, it was placed in a water bath for 45 minutes at 95°C. The content was then cooled in an ice bath for 5 minutes in the dark, and followed by the addition of n-butanol. After mixing and centrifugation, the supernatant was measured at 535 nm using a spectrophotometer (HP 8453, Hewlett Packard, Wilmington, DE, USA), and the difference was used for calculation of the TBARS concentration, which was expressed as nM/mg protein. The standard curve was prepared using 1,1,3,3-tetraethoxypropane from 0 to 50 µM/tube (R-square = 0.9894).

Analysis of hepatic GSH contents was performed using the method of Ellman [35] with modification; 100 µL of liver homogenate was mixed with the reaction mixture, which contained 0.1 ml of 0.2 M Tris–buffer (pH 8), 4 ml of methanol, and 0.1 ml of 0.01 M 5,5′-dithio-bis-2-nitrobenzoic acid. The solution was kept at room temperature for 15 minutes and then centrifuged for 30 minutes at 2,062 x g. The supernatant was measured at 415 nm using a Powerwave X microplate spectrophotometer (Biotek Instruments Inc., Winooski, VT, USA). The GSH contents were expressed as µM/mg protein.

Analysis of cytosolic superoxide dismutase (SOD) activity was performed using the OxiSelect Superoxide Dismutase Activity Assay Kit (Cell Biolabs, Inc., San Diego, CA, USA) [36].

2.7. Histological photographs of liver and adipose tissue

Histological photographs of liver and epididymal adipose tissue were examined using the paraffin method, under a light microscope. Fresh tissues were fixed immediately with 10% buffered formalin and paraffin-embedded sections were stained with hematoxylin and eosin. The stained sections were then examined by light microscopy.

2.8. Statistical analyses

All analyses were performed using the SPSS (statistical product and service solutions) 17.0 program. Values were expressed as the means ± standard error of the mean (SEM) and analyzed for significant difference by analysis of variance (ANOVA) and followed by Turkey-Kramer at a p < 0.05.

3. Results

3.1. Total phenolic and flavonoid contents and the effect on free radical scavenging activity of ELR

The contents of total phenolic and flavonoid in ELR were 195.4 ± 10.3 mg/g ELR and 9.4 ± 0.9 mg/g ELR, respectively. Analysis of the free radical scavenging activity of ELR was performed using the DPPH radical. EC50 of ELR was 69.7 ± 4.3 µg/ml and that of Vitamin C as a positive control was 5.8 ± 0.1 µg/ml (Table 3). These results suggest that ELR possesses free radical scavenging activity.

3.2. Effect of ELR on differentiation of pre-adipocytes into adipocytes

To examine the effect of ELR on differentiation of pre-adipocytes into adipocytes, pre-adipocytes were cultured under differentiation conditions for 14 days. As shown in Fig. 1, pre-adipocytes were differentiated into adipocytes and the differentiation was inhibited by ELR. The differentiated adipocytes showed intracellular lipid accumulation and the accumulated lipid droplets were examined by oil red O staining. Fat droplet formation was decreased by treatment with ELR and was almost completely blocked by treatment with 200 µg ELR/mL. According to results of the cell viability assay, pre-adipocytes treated with ELR at increasing doses for 72 h showed no cytotoxic effect (data not shown).

3.3. Effect of ELR on expression of adipocyte-specific proteins

RT-PCR was performed for examination of the effect of ELR on expression of adipogenesis transcription factor and terminal markers. Treatment with ELR resulted in reduction of the

| Table 3 – Total phenolic and flavonoid contents, and DPPH radical scavenging activity of ELR |
|-------------------------------------------------|------------------|
| Measurement                                      | Values           |
| Total phenolic contents                          | 195.4±10.3       |
| Total flavonoid contents                         | 9.4±0.9          |
| DPPH radical scavenging activity (EC50, µg/mL)   | 69.7±4.3         |
| ELR                                             | 5.8±0.1          |

Values are expressed as means±SEM. ELR, Ethanol extract of lotus root. EC50, efficient concentration of ELR decreasing initial DPPH concentration by 50%
mRNA level of PPAR-γ, a major adipogenic transcription factor. In addition, reduced expression of leptin and GLUT4, terminal markers, was observed in the presence of ELR (Fig. 2). These data suggest that ELR inhibited adipocyte differentiation through down-regulation of expression of PPAR-γ and subsequent expression of leptin and GLUT4.

3.4. Effect of ELR on body weight gain, diet intake, and food efficiency ratio

In order to study the anti-obesity effect of ELR in vivo, we fed a high-fat diet to rats for 14 weeks. Body weight gain in the HF group was higher than that of the N group, however, that of the HFR group was lower compared to the HF group (p < 0.05) (Fig. 3). Although the food intake (g/day) of the N group was higher, compared to the HF and HFR groups, no significant differences were observed in food intake (kcal/day) and water intake (g/day). The food efficiency ration of the HF group was significantly higher, compared to the N and HFR groups (Table 4).

3.5. Effect of ELR on organs and adipose tissues weights

No significant difference in the relative weight of liver, spleen, and kidney was observed among the groups (Table 5).
Relative weights of adipose tissues (E-fat and R-fat) of the HF group were significantly higher, compared to the N group, and those of the HFR group were significantly lower compared to the HF group (p < 0.05). Regarding histological appearance, epididymal adipocytes were larger in the HF group than in the N group. However, those in the HFR group were smaller than those in the N group and the HF group (Fig. 4). Hepatocytes in the three groups showed a similar histological appearance. From these results, it appears that ELR inhibits the increase of adipocyte size in rats fed a high-fat diet.

### 3.6. Effect of ELR on serum lipid, insulin, and adipokine levels

Results for serum lipid levels are shown in Table 6. The HF group showed a significant (p < 0.05) increase in serum TG, TC, and LDL-C levels compared with the N group. In contrast, ELR administration caused a significant (p < 0.05) decrease in serum TG and TC levels, compared to those in the HF group. However, no significant difference in serum HDL-C level was observed among all groups.

The HFR group showed a significant (p < 0.05) decrease in serum insulin levels compared with the N and HF groups (Table 7). Serum adiponectin levels tended to be lower in the HF group and were significantly (p < 0.05) decreased in the HFR group, compared to the N group. The HF group showed a significant (p < 0.05) increase in serum leptin levels compared with the N group, however, administration of ELR resulted in a significant (p < 0.05) decrease compared with that in the HF group.

### 3.7. Effect on hepatic TBARS and GSH contents, and cytosolic SOD activity

Results for TBARS and GSH contents and SOD activity are shown in Table 8. The hepatic TBARS content was higher in the HF group than in the N group, and ELR administration resulted in a significant (p < 0.05) decrease in hepatic TBARS contents, thus indicating that a high-fat diet increased oxidative stress and that ELR administration resulted in inhibition of oxidative stress in rats fed a high-fat diet. Lower hepatic GSH content was observed in the HF group than in the N group, and ELR administration resulted in a significant (p < 0.05) increase in hepatic GSH content. Significantly higher cytosolic SOD activity was observed in the HFR group (p < 0.05) than in the N and HF groups.

### 4. Discussion

Lotus root has commonly been consumed as a vegetable in many countries such as Korea, Japan, and China. Among various vegetables that include broccoli, parsley, eggplant,
garlic, and potato, the lotus root exhibits the strongest anti-oxidant activity [22]. In addition, the lotus root has a hypocholesterolemic effect [17], and hyperlipidaemia is potentially related to obesity. Until recently, a few studies reported the anti-obesity effect of lotus leaf [37] or seed [28], however, few studies on the root overall are even reported. Therefore, we investigated the anti-oxidant and anti-obesity effects of lotus root

in vitro and in vivo.

Solvents such as methanol, ethanol, acetone, hexane, water, or a combination are used for extraction of phytochemicals from plants. In general, compared with nonpolar solvents, polar organic solvents are commonly more effective for extraction, and these solvents affected the extraction yield and anti-oxidant activity [38,39]. According to a study on anti-oxidant activities in various extracts of lotus root, methanol extract had the highest extraction yield, total phenolic content, and anti-oxidant activity [38]. However, we used ethanol as a lotus root extract solvent for safety, and the extraction yield of ELR was 3.3 %.

Analysis of ELR was performed for determination of total phenolic content and total flavonoid content. Results for total phenolic and flavonoid content indicate that lotus root contains similar flavonoid content when compared with lotus seed (11.8 ± 0.5 mg CE/g), however, its phenolic content is considerably higher compared with that of lotus seed (23.2 ± 0.7 mg GAE/g) [28].

There are many methods for measurement of anti-oxidant activities. Among these methods, DPPH methods have frequently been used for assay of radical scavenging activities of natural sources. For analysis of DPPH radical scavenging activity, the efficient concentration of ELR that causes a decrease in initial DPPH concentration by 50% (EC50) was calculated. Anti-oxidant activity of ELR was lower compared to Vit C as a positive control but was higher compared to lotus seed ethanol extract (EC50: 224.5 ± 1.5 μg/mL). Total phenolic content does not include all anti-oxidants [40] and anti-oxidant activity was influenced by not only the concentration of anti-oxidant, but also the interaction among anti-oxidants.

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Table 6 – Effect of ELR on serum lipid profiles in rats fed a high-fat diet

<table>
<thead>
<tr>
<th>Serum lipid</th>
<th>Dietary group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal diet (mg/dl)</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>39.1±1.4 a</td>
</tr>
<tr>
<td>Total-cholesterol</td>
<td>84.2±3.8 a</td>
</tr>
<tr>
<td>LDL-cholesterol</td>
<td>41.6±3.1 a</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>31.8±1.7</td>
</tr>
</tbody>
</table>

Values are expressed as means±SEM (n=10). Values with different superscript within the row are significantly different at p<0.05, according to the ANOVA followed by Tukey-Kramer test.
Table 7 - Effect of ELR on serum insulin and adipokine level in rats fed a high-fat diet

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal diet</th>
<th>High-fat diet</th>
<th>High-fat diet + ELR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin (pg/ml)</td>
<td>728.6±127.8a</td>
<td>952.9±87.4a</td>
<td>432.3±44.6b</td>
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<tr>
<td>Adiponectin (pg/ml)</td>
<td>51.2±4.6b</td>
<td>42.3±2.7b</td>
<td>35.3±2.5b</td>
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<tr>
<td>Leptin (ng/ml)</td>
<td>3.2±0.3a</td>
<td>4.8±0.4a</td>
<td>3.2±0.2a</td>
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</tbody>
</table>

Values are expressed as means±SEM (n=10). Values with different superscript within the row are significantly different at p <0.05, according to the ANOVA followed by Tukey-Kramer test.

Table 8 - Effect of ELR on hepatic thiobarbituric acid reactive substance and glutathione content, and superoxide dismutase activity in rats fed a high-fat diet

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal diet</th>
<th>High-fat diet</th>
<th>High-fat diet + ELR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiobarbituric acid reactive substance contents (nmol/mg protein)</td>
<td>125.0±51.6a</td>
<td>355.4±71.3a</td>
<td>240.3±71.0b</td>
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<tr>
<td>Glutathione (nmol/mg protein)</td>
<td>119.5±3.1a</td>
<td>96.6±4.7b</td>
<td>111.4±6.1a</td>
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<tr>
<td>Superoxide dismutase activity (Inhibition rate (%))</td>
<td>35.8±5.3a</td>
<td>35.9±3.6b</td>
<td>41.9±2.7b</td>
</tr>
</tbody>
</table>

Values are expressed as means±SEM (n=10). Values with different superscript within the row are significantly different at p <0.05, according to the ANOVA followed by Tukey-Kramer test. Inhibition rate (%): (ODblank-ODsample)/(ODblank)×100.
in obese rodent models [54]. A single injection of adiponectin in normal or ob/db mice leads to a decrease in the basal glucose level. Serum adiponectin may have contributed to suppression of hepatic expression of lipogenic and inflammatory genes in lotus diet-fed db/db mice [55], and serum adiponectin level also showed a positive correlation with HDL-C and systolic blood pressure. Accordingly, it appears that adiponectin has properties of anti-atherosclerosis and anti-insulin resistance [56,57]. However, the previous study reported that no differences in blood adiponectin levels were observed among groups in obese mice [58], and in this study, serum adiponectin levels of the HFR group did not show an increase compared to the HF group. No consistent association was observed in rodent models.

Leptin is an adipokine secreted from adipocytes, and insulin is a key hormone for the regulation of food intake and energy expenditure [59]. When rats were fed a high-fat diet, the serum leptin level was increased and administration of ELR resulted in decreased levels of serum leptin and insulin compared to rats without ELR. Hyperinsulinaemia in obesity and leptin and insulin levels are reported to show high correlations with body weight and visceral fat. Weight reduction was reported to cause a decrease in serum leptin levels [60]. Leptin caused a decrease in energy intake; however, a high level of leptin in obese subjects did not suppress appetite due to leptin resistance. In this study, although no significant difference in food intake was observed between the HF and HFR groups, reduced weight and size of epididymal fat was observed in the HFR group. Therefore, ELR administration may cause a decrease in lipid accumulation in human adipocytes, adipose tissue weights, and serum leptin levels in rats fed a high-fat diet.

Lotus root contains fatty acid, phytosterol [61], alkaloids (e.g. nuciferine, liensinine, isoliensinine, neferine, and dauerine), and flavonoids (e.g. catechin, rutin, isoquercitrin, and astragcline). Isolation of tryptophan from aqueous extract of lotus root [22] has been reported, and the condensed tannins contained in lotus root alleviated hepatic steatosis in db/db mice [62].

This study had some limitations. First, we examined PPAR-γ, GLUT4, and leptin in human pre-adipocytes using RT-PCR; however, to elucidate the exact mechanism of ELR administration may cause a decrease in lipid accumulation in human adipocytes, adipose tissue weights, and serum leptin levels in rats fed a high-fat diet.

Based on our study, we accept our hypothesis since ELR exhibited an anti-adipogenic effect in human pre-adipocytes and anti-obesity and anti-oxidant effects in rats fed a high-fat diet. These results suggest that lotus root could be used as a functional and nutraceutical ingredient in obesity-related diseases.

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REFERENCES
