Anti-obesity and hypolipidaemic effects of *Nelumbo nucifera* seed ethanol extract in human pre-adipocytes and rats fed a high-fat diet

Jeong Soon You,a Yun Ju Lee,a Kyung Soo Kim,b Sung Hoon Kimc and Kyung Ja Changa

Abstract

BACKGROUND: We conducted this investigation in order to examine the anti-obesity and hypolipidaemic effects of *Nelumbo nucifera* seed ethanol extract (NSEE) *in vitro* and *in vivo*.

METHODS: To study the anti-obesity effect of NSEE *in vitro* and *in vivo*, human pre-adipocytes were treated with NSEE, and male Sprague–Dawley rats were fed with a normal diet and a high-fat diet with or without NSEE, respectively.

RESULTS: *In vitro* treatment with NSEE resulted in inhibition of lipid accumulation and decreased expression of peroxisome proliferator-activated receptor gamma (PPARγ), glucose transporter 4 (GLUT4), and leptin in cultured human adipocytes, indicating that it inhibited the differentiation of pre-adipocytes into adipocytes. Administration of NSEE resulted in significantly reduced body weight gain and adipose tissue weights in rats. Serum triglyceride and leptin level of the high-fat diet + NSEE group was significantly lower, compared to the high-fat group.

CONCLUSION: These results demonstrate an inhibitory effect of NSEE on adipogenesis. In addition, NSEE had a beneficial effect, reducing adipose tissue weights, ameliorating blood lipid profile, and modulating serum leptin level in rats fed a high-fat diet. Therefore, we suggest that lotus seed has a potential to be developed as an effective agent against obesity-related diseases.

© 2013 Society of Chemical Industry

Keywords: anti-obesity; hypolipidaemic; *Nelumbo nucifera* seed; human pre-adipocyte; high-fat diet

INTRODUCTION

Obesity is associated with many metabolic diseases, including cardiovascular disease, diabetes mellitus, high blood pressure, atherosclerosis, various cancers, and hyperlipidaemia.1,2 Obesity is caused by excessive fat in the body, and excessive fat accumulation results in increased triglyceride concentration in blood. In addition, it also leads to insulin resistance related metabolic syndrome.1 Use of various medicinal plants in the treatment of obesity has been reported.3,4

*Nelumbo nucifera* has been widely cultivated and used as a food, medicine, and ornamental in India and eastern Asia, including China, Japan, Thailand, Vietnam and Korea. *Nelumbo nucifera* is an eco-friendly crop that can be grown without herbicides and pesticides and has powerful water purification ability. *Nelumbo nucifera* has been reported to possess pharmacological activities, such as antioxidant,5,6 hepatoprotective,7,8 antibacterial,9 hypolipidaemic,10 anti-obesity,11,12 and hypoglycaemic13 effects. Among the different parts, alkaloid isolated from lotus leaf inhibited 3T3-L1 pre-adipocyte differentiation and improved high-fat diet-induced obesity and body fat accumulation in rats.11

*Nelumbo nucifera* seed has been used in traditional Korean medicine as a treatment for insomnia, anxiety and depression in women following the menopause. However, the seed of *Nelumbo nucifera* has only been reported to possess antioxidant activity14 and hepatoprotective and free radical scavenging activity,15 and to act as a chemo-preventer through reduction of excess nitric oxide.16 The anti-obesity and hypolipidaemic effect of *Nelumbo nucifera* seed ethanol extract (NSEE) has not been studied.

Therefore, this study was conducted in order to evaluate the anti-obesity and hypolipidaemic effects of NSEE using *in vitro* and *in vivo* experiments. For *in vitro* experiments, human pre-adipocytes...
were differentiated and lipid accumulation in these cells was measured by oil red O staining. For in vivo experiments, NSEE was administrated orally to rats fed a high-fat diet; we measured adipose tissue weight, serum lipid level, serum insulin, and adipokine level.

MATERIALS AND METHODS
Preparation of Nelumbo nucifera seed ethanol extract
Frozen lotus seed (1.4 kg) purchased from Siheung (Kyunggi-do, Korea) was ground and extracted with 7 L of 700 mL L\(^{-1}\) ethanol at 50 °C in a water bath for 2 h. After vacuum filtration, the extract was combined and evaporated under reduced pressure, leaving a dry extract (96.5 g). The extract ratio was 68.3 g kg\(^{-1}\). This extract was stored in a desiccator under reduced pressure until application.

Determination of total phenolic and flavonoid content of Nelumbo nucifera seed ethanol extract
Analysis of total phenolic contents was performed using the Folin–Ciocalteu assay.\(^{17}\) NSEE (2 mg mL\(^{-1}\), 0.1 mL) was mixed with distilled water (2 mL) and Folin–Ciocalteu reagent (1 mL). After 5 min, 200 g L\(^{-1}\) aqueous sodium carbonate solution (5 mL) was added to the mixture, followed by incubation in the dark for 60 min. Absorbance was measured at 735 nm. The total phenolic contents were determined using gallic acid as a calibration standard and the results were expressed as milligrams of gallic acid equivalents per gram of sample.

Total flavonoid contents were determined using the colorimetric method, which was modified from the method of Lin et al.\(^{17}\) The sample (2 mg mL\(^{-1}\), 0.6 mL) was mixed with distilled water (3.75 mL) and 5% aqueous sodium nitrite solution (0.225 mL). After 6 min, 10% aqueous aluminium chloride solution (0.45 mL) was added; after 5 min, sodium hydroxide solution (1 mol L\(^{-1}\), 1.5 mL) was added, followed by addition of distilled water until the total volume reached 7.5 mL. Absorbance was measured at 510 nm. The total flavonoid contents were determined using catechin as a calibration standard at various concentrations (0–800 µg mL\(^{-1}\)). The results were expressed as milligrams of catechin equivalents per gram of sample.

Pre-adipocyte cell culture and differentiation into adipocytes
Human pre-adipocytes were purchased from Cell Applications (San Diego, CA, USA) and maintained using a pre-adipocyte growth medium kit (Cell Applications). Pre-adipocytes were seeded into six-well plates (1.8 × 10\(^5\) cell per well in 2 mL of medium) and cultured until confluent. For differentiation, the culture medium was changed to adipocyte differentiation medium (Cell Applications) and cultured for 2 weeks by changing the medium every 3 days in the presence or absence of NSEE at different concentrations (0, 25, 50, and 100 µg mL\(^{-1}\)).

Cell viability
For assessment of cell viability of NSEE, a cell proliferation assay was performed using a cell counting kit-8 (Dojindo Lab., Tokyo, Japan). Briefly, the cells were seeded in 96-well plates at a density of 2 × 10\(^4\) cells per well. After incubation for 24 h, the cells were exposed to various concentrations of NSEE in a volume of 100 µL. After incubation for 72 h, 10 µL of WST-8 dye (Dojindo Lab.) was added to each well, followed by incubation for 3 h at 37°C. The optical density was read at 450 nm in an Emax microplate reader (Molecular Devices, Sunnyvale, CA, USA). Results were calculated as the percentage of viable cells in the NSEE-treated group compared to the control group.

Oil red O staining
Oil red O staining was performed for examination of lipid.\(^{18}\) After removal of the medium, cultured cells were washed twice with phosphate-buffered saline and fixed in 100 mL L\(^{-1}\) formaldehyde for 1 h. Formaldehyde was removed and cells were rinsed twice with deionised water. The cells were stained with oil red O (Sigma, St. Louis, MO, USA) solution (1.8 mg mL\(^{-1}\) in isopropanol) for 20 min at room temperature. Microscopy images (Olympus, Tokyo, 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).

RNA isolation and reverse transcription–polymerase chain reaction
Total RNA was isolated from human adipocytes using Trizol (Invitrogen, Carlsbad, CA, USA). Complementary DNA synthesis was performed using 1 µg total RNA in 20 µL reverse transcription reaction mixture containing 5 mmol L\(^{-1}\) MgCl\(_2\), 10 × RT buffer, 1 mmol L\(^{-1}\) dNTP, 1 U µL\(^{-1}\) RNase inhibitor, 0.25 U µL\(^{-1}\) AMV reverse transcriptase, and 2.5 µmol L\(^{-1}\) random 9-mers, as described previously.\(^{19}\) The PCR conditions for leptin, glucose transporter 4 (GLUT-4), peroxisome proliferator-activated receptor gamma (PPAR\(\gamma\)\(_{p}\)) and \(\beta\)-actin were as follows: 28 cycles for PPAR\(\gamma\)\(_{p}\) at 95°C for 45 s, 55°C for 45 s, and 72°C for 45 s; 35 cycles for leptin at 95°C for 45 s, 59°C for 45 s, and 72°C for 45 s; 35 cycles for GLUT4 at 95°C for 45 s, 62°C for 45 s, and 72°C for 45 s. The RT-PCR products were electrophoresed in 15 g L\(^{-1}\) agarose gels containing ethidium bromide, and the bands were visualised under a Bio-Rad universal hood II (Bio-Rad Laboratories, Segrate, Italy). The primers were synthesised by Bioneer Co. Ltd (Seoul, Korea) (Table 1).

Preparation of animals and diet
Four-week-old male Sprague–Dawley rats were purchased from Koatech (Anseong, Korea). All rats were kept in laboratory animal housing at Kyung Hee University with a constant 12 h light and dark cycle (07.00 a.m. to 07.00 p.m.), controlled temperature (22 ± 1°C) and humidity (55 ± 10%). All the procedures of animal care were conducted in accordance with the guidelines outlined by the Experimental Animal Ethics Committee of Kyung Hee University. Following 1 week of acclimatisation with a pelleted commercial diet, rats were fed separately with a normal diet (N group) and a high-fat diet. After the first 7 weeks, rats fed a high-fat diet were

<table>
<thead>
<tr>
<th>Table 1. Sequences for primers set by RT-PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gene name</strong></td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>PPAR(\gamma)(_{p})</td>
</tr>
<tr>
<td>Leptin</td>
</tr>
<tr>
<td>GLUT4</td>
</tr>
<tr>
<td>(\beta)-Actin</td>
</tr>
<tr>
<td>PPAR(\gamma)(_{p}), peroxisome proliferator-activated receptor gamma; GLUT4, glucose transporter 4.</td>
</tr>
</tbody>
</table>
the experimental diet was based on AIN-93,20 as shown in Table 2. Body weight was measured once per week. The composition of groups. Food and water intake was measured twice per week and of saline solution was administered orally to rats in the N and HF groups. Food and water intake was measured twice per week and body weight was measured once per week. The composition of the experimental diet was based on AIN-93,20 as shown in Table 2.

### Table 2. Composition of the experimental diet

<table>
<thead>
<tr>
<th>Component</th>
<th>Normal diet (g kg⁻¹ diet)</th>
<th>High-fat diet (g kg⁻¹ diet)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>200.0</td>
<td>200.0</td>
</tr>
<tr>
<td>Corn starch</td>
<td>447.486</td>
<td>155.036</td>
</tr>
<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 mixture</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>AIN-93 mineral mixture</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>
</tbody>
</table>

TBHQ, tert-butylhydroquinone.

randomly divided into two sub-groups [high-fat diet (HF group); high-fat diet + NSEE (HFS group)]. NSEE (400 mg kg⁻¹ day⁻¹) was administered orally to rats in the HFS group and the same amount of saline solution was administered orally to rats in the N and HF groups. Food and water intake was measured twice per week and body weight was measured once per week. The composition of the experimental diet was based on AIN-93,20 as shown in Table 2.

### Sampling and chemical analysis

After 14 weeks, the animals were fasted for 12 h before sacrifice. Blood was collected from the heart and serum was obtained by centrifugation at 1650 × g for 20 min. The liver, kidney, spleen, epididymal fat (E-fat) and retroperitoneal fat (R-fat) were weighed. Some E-fat was removed from the rats for histological photography. The serum was stored at −70°C until application.

Analysis of serum triglyceride (TG) and total cholesterol (TC) levels was 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 10 min at 800 × g, followed by analysis for HDL-C using the same method used with TC. Serum low-density lipoprotein cholesterol (LDL-C) value was calculated using the Friedewald formula,21 as follows:

$$LDL-C = TC - (HDL-C + TG/5)$$

Assayed chemistry control (Bio-Rad Laboratories, Irvine, CA, USA) was used for calibration and all of the results were expressed as mg dL⁻¹ serum.

Analysis of serum adiponectin level was performed using a rat single plex adiponectin 96-well plate MILLIPLEX map kit (Millipore Corporation, Billerica, MA, USA) and analysis of serum insulin and leptin levels was performed using a rat serum adipokine 96-well plate MILLIPLEX map kit (Millipore) through luminex 100 (MiraBio, Inc., Alameda, CA, USA).

### Histopathology

Histological photographs of epididymal adipose tissue and liver were examined under a light microscope using the paraffin method. Fresh tissues were fixed immediately with 10% buffered formalin, and paraffin-embedded sections were stained with haematoxylin and eosin (H&E).

### Statistical analysis

Data were expressed as the mean ± standard error of the mean (SEM) and one-way analysis of variance followed by Duncan’s multiple range tests at $P < 0.05$ were used for analysis for significant difference. All analyses were performed using the SPSS 17.0 program.

### RESULTS

#### Contents of total phenolics and flavonoids from of *Nelumbo nucifera* seed ethanol extract

The contents of total phenolics and flavonoids in NSEE were 23.24 ± 0.65 mg g⁻¹ and 11.81 ± 0.53 mg g⁻¹, respectively.

#### In vitro effects of *Nelumbo nucifera* seed ethanol extract on differentiation of pre-adipocytes into adipocytes

To examine the anti-adipogenic effect of NSEE on human adipocyte differentiation, pre-adipocytes were cultured in differentiation media for 14 days with various doses of NSEE (0, 25, 50 and 100 µg mL⁻¹). Lipid accumulation was measured by oil red O staining. As shown in Fig. 1, NSEE inhibited adipocyte differentiation in human adipocytes and lipid accumulation was almost completely blocked by treatment with 100 µg mL⁻¹ NSEE.

Pre-adipocytes were examined in order to investigate the cell viability of NSEE. NSEE ranging from 0 to 200 µg mL⁻¹ was applied to pre-adipocytes. NSEE showed no cytotoxic effect on pre-adipocytes after 3 days at increasing doses (data now shown).

To elucidate the molecular mechanisms by which it inhibited the adipogenesis, the expression level of adipogenesis transcription factor and terminal markers was examined. Expression of PPARγ, GLUT4 and leptin was significantly reduced in the presence of NSEE in a dose-dependent manner (Fig. 2). These data suggested that treatment with NSEE resulted in down-regulated expression of PPARγ and subsequent expression of GLUT4 and leptin, resulting in inhibition of adipocyte differentiation.

#### The effect of *Nelumbo nucifera* seed ethanol extract on body weight, diet intake, and food efficiency ratio

To examine the anti-obesity effect of NSEE, we fed rats a high-fat diet for 14 weeks. At the end of the experiment, rats fed a high-fat diet had a significantly higher body weight gain than rats fed a normal diet. However, administration of NSEE resulted in suppression of high-fat diet induced body weight gain (Table 3).

No significant difference was observed in food intake (in kilocalories), although the food intake (in grams) of the HF and HFS groups was lower, compared to the N group. The food efficiency ratio (FER) of the N group was significantly lower, compared to the HF and HFS groups.

#### The effect of *Nelumbo nucifera* seed ethanol extract on organs and adipose tissues

No significant differences in the relative weights of liver, spleen and kidney were observed among the groups (Table 4). The 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 HFS group were significantly lower, compared to the HF group. Hepatocytes showed a similar histological appearance
among three groups. Epididymal adipocytes showed an irregular histological appearance in the HF group, compared to the N group. However, this morphological change was not observed in the HFS group (Fig. 3). Therefore, NSEE may inhibit the increase of body fat induced in rats fed a high-fat diet.

**The effect of of *Nelumbo nucifera* seed ethanol extract on serum lipid, insulin and adipokine levels**

Serum lipid levels are shown in Table 5. The increased serum TG level in the HF group was significantly decreased in the HFS group. The serum TC and LDL-C levels were significantly increased in the HF group, compared to the N group; however, no significant difference was observed between the HF group and the HFS group.

The serum adiponectin level was significantly decreased in the HFS group, compared to the N group, although serum insulin level did not differ significantly among the groups (Table 6). The increased serum leptin level in the HF group was significantly decreased in the HFS group.

**DISCUSSION**

*Nelumbo nucifera* seed has been used in traditional Korean medicine as a treatment for insomnia, anxiety and depression in women following the menopause. However, the anti-obesity and hypolipidaemic effect of NSEE has not been studied. Obesity is a very important risk factor for metabolic diseases and the prevalence of obesity has increased over the past few decades. This study investigated the anti-obesity effect of NSEE in vitro and in vivo using human pre-adipocytes and rats fed a high-fat diet, respectively.

Obesity means excessive fat accumulation in the body; fat is stored primarily in adipose tissue, which consists of adipocytes, pre-adipocytes, vascular smooth muscle cells, macrophages, endothelial cells, fibroblasts and lymphocytes. Both hyperplastic (increase in number of adipocytes) and hypertrophic (increase in cell volume) mechanisms are involved in excessive growth of adipose tissue in obesity. The cell volume of adipocytes is associated with accumulation of TG. Therefore, reduction of TG accumulation by inhibition of adipogenesis is a very important mechanism in in vitro anti-obesity activity.

Human pre-adipocytes were cultured for 14 days in order to examine the effects of NSEE on differentiation of human pre-adipocytes into adipocytes. Differentiation was inhibited by NSEE and the results from oil red O staining showed that NSEE inhibited lipid accumulation during adipocyte differentiation. The result of cell viability suggested that the inhibitory effect of
genes, such as GLUT4, leptin, fatty acid synthetase, and others.25

transcription factors drive the expression of adipocyte marker binding protein-3 days with NSEE at various concentrations (0–100 µg mL⁻¹) for 14 days in differentiation medium. The cells were prepared for semi-quantitative RT-PCR. The data shown are representative of three independent experiments and similar results were obtained from all three.

NSEE on differentiation and lipid accumulation was not due to cytotoxicity.

The cells during adipogenesis accumulate large fat droplets.24 Then, an upstream regulator such as sterol-regulatory element binding protein-1c (SREBP1c) regulates the expression of adipogenic target genes, such as carnitine palmitoyl transferase-1 (CPT-1) and thiolase.

In this study, we also used rats fed a high-fat diet in order to examine the anti-obesity effects of NSEE in vivo. Treatment with NSEE did not produce any signs of acute toxicity; the LD₅₀ (lethal dose for 50% of the animals) and approximate lethal dose of NSEE in both female and male ICR mice were considered as greater than 5000 mg kg⁻¹ body weight. Body weight gain and adipose tissue weights in the HFS group were lower, compared to the HF group. Treatment with NSEE appeared to result in a decrease in size of epididymal adipocytes, which was increased in the HF group. Interestingly, treatment with NSEE resulted in decreased body weight gain, epididymal fat tissue, and retroperitoneal fat tissue weight; however, food intake (kcal day⁻¹) did not differ significantly among groups. At once, NSEE did not affect food intake and may have affected adipogenesis and lipid accumulation in adipocytes. This is supported by results of our in vitro study, in which NSEE had an anti-adipogenic effect on human pre-adipocytes differentiated into adipocytes.

In this study, we also used rats fed a high-fat diet in order to examine the anti-obesity effects of NSEE in vivo. Treatment with NSEE did not produce any signs of acute toxicity; the LD₅₀ (lethal dose for 50% of the animals) and approximate lethal dose of NSEE in both female and male ICR mice were considered as greater than 5000 mg kg⁻¹ body weight. Body weight gain and adipose tissue weights in the HFS group were lower, compared to the HF group. Treatment with NSEE appeared to result in a decrease in size of epididymal adipocytes, which was increased in the HF group. Interestingly, treatment with NSEE resulted in decreased body weight gain, epididymal fat tissue, and retroperitoneal fat tissue weight; however, food intake (kcal day⁻¹) did not differ significantly among groups. At once, NSEE did not affect food intake and may have affected adipogenesis and lipid accumulation in adipocytes. This is supported by results of our in vitro study, in which NSEE had an anti-adipogenic effect on human pre-adipocytes differentiated into adipocytes.

NSEE-treated cells expressed low levels of PPARγ, compared to cells not treated with NSEE. Furthermore, the expression of GLUT4 was significantly reduced in the presence of NSEE. These results suggest that NSEE down-regulates expression of PPARγ, resulting in inhibition of adipocyte differentiation.

In this study, we also used rats fed a high-fat diet in order to examine the anti-obesity effects of NSEE in vivo. Treatment with NSEE did not produce any signs of acute toxicity; the LD₅₀ (lethal dose for 50% of the animals) and approximate lethal dose of NSEE in both female and male ICR mice were considered as greater than 5000 mg kg⁻¹ body weight. Body weight gain and adipose tissue weights in the HFS group were lower, compared to the HF group. Treatment with NSEE appeared to result in a decrease in size of epididymal adipocytes, which was increased in the HF group. Interestingly, treatment with NSEE resulted in decreased body weight gain, epididymal fat tissue, and retroperitoneal fat tissue weight; however, food intake (kcal day⁻¹) did not differ significantly among groups. At once, NSEE did not affect food intake and may have affected adipogenesis and lipid accumulation in adipocytes. This is supported by results of our in vitro study, in which NSEE had an anti-adipogenic effect on human pre-adipocytes differentiated into adipocytes.

Figure 2. Effect of NSEE on mRNA expression of PPARγ, leptin and GLUT4 in human adipocytes. Differentiating human adipocytes were treated every 3 days with NSEE at various concentrations (0–100 µg mL⁻¹) for 14 days in differentiation medium. The cells were prepared for semi-quantitative RT-PCR. The data shown are representative of three independent experiments and similar results were obtained from all three.

Table 3. Effect of Nelumbo nucifera seed ethanol extract (NSEE) on relative organ weights (g kg⁻¹ body weight) of rats in the three dietary groups

Table 4. Effect of Nelumbo nucifera seed ethanol extract (NSEE) on mRNA expression of PPARγ, leptin and GLUT4 in human adipocytes. Differentiating human adipocytes were treated every 3 days with NSEE at various concentrations (0–100 µg mL⁻¹) for 14 days in differentiation medium. The cells were prepared for semi-quantitative RT-PCR. The data shown are representative of three independent experiments and similar results were obtained from all three.

Figure 3. Light micrography of (A) epididymal adipocytes and (B) hepatocytes in rats fed a high-fat diet. Representative pictures of haematoxylin and eosin-stained sections of epididymal adipocytes from rats fed a normal diet (N) or high-fat diet + NSEE (HFS) show smaller size of adipocytes, compared with those of rats fed a high-fat diet (HF). Scale bar = 20 µm.
High fat intake induces hyperlipidaemia, which can lead to various cardiovascular diseases, such as atherosclerosis and coronary heart disease, both of which are common causes of death in modern society. In this study, rats in the HF group exhibited higher levels of TG and HDL-C, compared to non-obese subjects. Obesity subjects had higher plasma leptin levels, compared to lean subjects, and the blood leptin level is highly correlated with body weight and TG stores in adipocytes. In this study, rats fed a high-fat diet, compared to the N group. Although body weight gain and TG level were lower in the HFS group, compared to the HF group, the adiponectin level of the HFS group was not higher than that of the HF group. A previous article reported that administration of Citrus depressa Hayata in high-fat diet-induced obese mice resulted in an anti-obesity effect, including lowering of body weight, adipose tissue weight, and TG level; however, no change in adiponectin level was observed. No consistent association was observed in rodent models.

In this study, serum adiponectin level tended to be lower in rats fed a high-fat diet, compared to the N group. Although body weight gain and TG level were lower in the HFS group, compared to the HF group, the adiponectin level of the HFS group was not higher than that of the HF group. A previous article reported that administration of Citrus depressa Hayata in high-fat diet-induced obese mice resulted in an anti-obesity effect, including lowering of body weight, adipose tissue weight, and TG level; however, no change in adiponectin level was observed. No consistent association was observed in rodent models.

**Table 5. Effect of *Nelumbo nucifera* seed ethanol extract (NSEE) on serum lipid profiles of rats in the three dietary groups**

<table>
<thead>
<tr>
<th>Serum lipid</th>
<th>Normal diet</th>
<th>High-fat diet</th>
<th>High-fat diet + NSEE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride (mg dL⁻¹)</td>
<td>39.07 ± 1.39a</td>
<td>51.31 ± 3.13b</td>
<td>31.27 ± 1.78ab</td>
</tr>
<tr>
<td>Total cholesterol (mg dL⁻¹)</td>
<td>84.21 ± 3.76a</td>
<td>93.68 ± 2.52b</td>
<td>85.50 ± 2.35ab</td>
</tr>
<tr>
<td>LDL-cholesterol (mg dL⁻¹)</td>
<td>41.62 ± 3.05a</td>
<td>52.32 ± 1.98b</td>
<td>50.52 ± 2.26b</td>
</tr>
<tr>
<td>HDL-cholesterol (mg dL⁻¹)</td>
<td>31.83 ± 1.66</td>
<td>30.75 ± 1.54</td>
<td>30.50 ± 1.62</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM. Values with different superscript within the row were significantly different at P < 0.05 by Duncan’s multiple range test.

**Table 6. Effect of *Nelumbo nucifera* seed ethanol extract (NSEE) on serum insulin and adipokine levels of rats in the three dietary groups**

<table>
<thead>
<tr>
<th>Insulin/adipokine</th>
<th>Normal diet</th>
<th>High-fat diet</th>
<th>High-fat diet + NSEE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin (pg mL⁻¹)</td>
<td>728.60 ± 127.82</td>
<td>952.31 ± 87.37</td>
<td>808.00 ± 108.50</td>
</tr>
<tr>
<td>Adiponectin (µg mL⁻¹)</td>
<td>51.24 ± 4.64a</td>
<td>42.33 ± 2.68ab</td>
<td>35.68 ± 3.94b</td>
</tr>
<tr>
<td>Leptin (ng mL⁻¹)</td>
<td>3.18 ± 0.27a</td>
<td>4.81 ± 0.35b</td>
<td>3.13 ± 0.34b</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM. Values with different superscript within the row were significantly different at P < 0.05 by Duncan’s multiple range test.

Adipose tissue not only stores excess energy but also releases physiologically active mediators known as adipokines, such as adiponectin and leptin. Adiponectin, which is produced by adipocytes, possesses antihyperglycaemic, anti-atherogenic, and anti-inflammatory properties. The adiponectin level is negatively correlated with body mass index, glucose, insulin and triglyceride levels and positively correlated with HDL-C level. In addition, plasma adiponectin levels were reduced in obese subjects, compared to non-obese subjects, and weight loss and therapy with thiazolidinediones resulted in increased endogeneous secretion of adiponectin in humans.

**CONCLUSIONS**

These results demonstrate an inhibitory effect of NSEE on adipogenesis. In addition, NSEE had a beneficial effect, reducing adipose tissue weights, ameliorating blood lipid profile, and modulating serum leptin level in rats fed a high-fat diet. Therefore, we suggest that lotus seeds have a potential to be developed as an effective agent against obesity-related diseases.

**ACKNOWLEDGEMENTS**

This research was supported by the Globalization of Korean Foods R&D program, funded by the Ministry of Food, Agriculture, Forestry and Fisheries, Republic of Korea.
REFERENCES


