Inhibitory Effect of a Cirsium setidens Extract on Hepatic Fat Accumulation in Mice Fed a High-Fat Diet via the Induction of Fatty Acid $\beta$-Oxidation

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Cirsium setidens is a perennial medicinal herb that is rich in flavonoids. We investigated in this study the effect of a C. setidens ethanol extract (CSE) on the development of nonalcoholic fatty liver in mice fed a high-fat diet (HF). C57BL/6J mice were fed either a control diet (CON) or HF for 8 weeks, and then fed CON, HF, or HF with 100 mg/kg of BW CSE (HF+CSE) for an additional 7 weeks. The final body weight and adipose tissue weight of the mice in the HF+CSE group were significantly lower than those in the HF group. CSE also markedly diminished both the lipid droplets in the liver tissues and decreased the hepatic and serum triglycerides (TG) concentrations. CSE strongly increased the hepatic mRNA levels of carnitine palmitoyltransferase (CPT1) and medium-chain acyl-CoA dehydrogenase (MCAD), the fatty acid $\beta$-oxidation enzymes. The hepatic levels of phosphorylated-AMP-activated protein kinase (AMPK) were significantly higher in the HF+CSE group than in the HF group. These results suggest that CSE inhibited hepatic fat accumulation by up-regulating the expression of the fatty acid $\beta$-oxidation genes.

Key words: Cirsium setidens; nonalcoholic fatty liver; fatty acid oxidation; mouse

Nonalcoholic fatty liver disease (NAFLD) is a serious health problem in both industrialized and developing countries.1) NAFLD is considered to be a hepatic manifestation of the metabolic syndrome, and the pathogenesis of NAFLD is closely related with obesity and dysregulated lipid metabolism. In the early stage of NAFLD, triglycerides are accumulated in the cytoplasm of hepatocytes, which leads to hepatic steatosis or fatty liver. It is thought that the hepatic fat accumulation predisposes the liver tissue to be more susceptible to additional insults such as oxidative stress and inflammation, exacerbating liver injury.2) The prevalence of NAFLD and obesity continues to increase worldwide, but effective strategies to prevent or treat these diseases are still not available.3) The need to find effective substances from dietary sources to mitigate the development of nonalcoholic fatty liver and obesity is therefore warranted.

Cirsium setidens is a wild perennial herb of the Compositae family which has been widely used in traditional medicine for treating hemorrhage, hypertension, and diabetes. It has been shown that C. setidens contains various biologically active flavonoids including pectolinarin, luteolin, and apigenin.4) Previous studies have reported that the C. setidens extract and its components had a number of health benefits; for example, a C. setidens extract at a dose of 100 mg/kg of BW for two weeks to mice showed antioxidative and hepatoprotective activities against $\alpha$-galactosamine-induced liver damage.5) The butanol fraction of C. setidens reduced CCl4-induced liver injury to rats by increasing the transcript levels of glutathione peroxidase and superoxide dismutase.6) In addition, Martinez-Vazquez et al.7) have reported on the analgesic and anti-inflammatory activities of pectolinarin isolated from C. subcoriaceum. Liao et al.8) have recently shown that pectolinarin isolated from C. japonicum DC had an anti-diabetic effect on diabetic rats established by injecting with streptozotocin and subsequent feeding with a high-carbohydrate/high-fat diet. However, the potential effect of C. setidens against non-alcoholic fatty liver and obesity, and its related mechanisms have not been fully investigated. We examined in the current study the effect of standardized ethanol extracts of C. setidens on the development of non-alcoholic fatty liver and obesity in mice fed a high-fat diet. The effects of C. setidens on the expression of genes involved in lipid metabolism were also investigated.

Materials and Methods

Preparation and standardization of the Cirsium setidens extract (CSE), and the component analysis of CSE. Cirsium setidens was purchased from a local farm (Kangwon-do, Korea). The freeze-dried Cirsium setidens sample was extracted with 70% ethanol for 1 h by stirring and left for 3–4 h at room temperature. The extract was then decanted into another flask, after which the extraction was repeated twice more. The extract was passed through 8-μm filter paper (Whatman, Maidstone, UK) and then concentrated under vacuum to

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Abbreviations: ACC, acetyl-CoA carboxylase; ACO, acetyl-CoA oxidase; AMPK, AMP-activated protein kinase; CON, control diet; CPT, carnitine palmitoyltransferase; CSE, Cirsium setidens ethanol extract; FAS, fatty acid synthase; HF, high-fat diet; MCAD, medium-chain acyl-CoA dehydrogenase; NAFLD, non-alcoholic fatty liver; PPAR, peroxisome proliferator-activated receptor; SREBP1, sterol regulatory element binding transcription factor; TG, triglyceride
obtain CSE. The average yield of CSE from dried Cirsium setidens was 18.3%.

Since pectolinarin and its aglycon pectolinarigenin are known to be abundant in C. setidens, we measured the concentrations of pectolinarin and pectolinarigenin in CSE by using an HPLC analysis. CSE was first extracted for 25 min with 100% methanol at 60°C while shaking, and then passed through a syringe filter (0.2 μm, Millipore Co., Bedford, MA, USA). The apparatus used was a Jasco (Tokyo, Japan) HPLC system with a PU-2080 Plus gradient pump equipped with a degasser, an AS-2075 Plus autosampler, and a MD-2010 Plus multiwavelength detector. Data were collected with Jasco Chrompass software. A comparative analysis was carried out by using a SunFire (Waters, Milford, MA, USA) C18 column (4.6 mm i.d. × 250 mm, 5-μm pore size). The mobile phase was a binary eluant of (A) 0.5% phosphoric acid in water and (B) methanol under gradient conditions. The linear gradient elution program was set as follows: eluent A was decreased from 75% to 45% over 25 min, maintained at 45% for 30 min, decreased from 45% to 0 over 20 min, maintained at 0 for 3 min, increased from 0 to 75% in the next 5 min, and equilibrated for 7 min before the next injection. The solvent flow rate was 0.5 mL/min, and the eluted pectolinarin and pectolinarigenin were detected at 332 nm. The injection volume was 10 μL, and the column temperature was maintained at 25°C. The contents of pectolinarin and pectolinarigenin in CSE used for the animal study are shown in Table 1.

We also determined the total polyphenol and carbohydrate contents by Folin-Ciocalteu and phenol-H2SO4 methods, using gallic acid and glucose as the respective standards. The total protein, lipid and ash contents were measured according to the Korean Food Standards Codex official methods. The composition of CSE was 16.4% carbohydrate, 39.4% lipid, 11.5% protein, and 16.4% ash. The total polyphenol content of CSE was 10.8%.

**Animals and diets.** All animals were approved by the Institutional Animal Care and Use Committee at Kyung Hee University. Male C57BL/6 mice (4 weeks old, SLC, Japan) were housed in a room with controlled temperature (21–23°C), humidity (55–60%) and lighting (12-h light/dark cycle), and provided free access to a rodent diet and water. After acclimatization for 1 week, the animals were assigned by weight-matching to three groups. One group was fed a control diet (10% kcal from fat) purchased from Research Diets (New Brunswick, NJ, USA), and two groups were fed a high-fat diet (45% kcal from fat; Research Diets) for eight weeks to induce obesity. The mice then continued to be fed a control diet (CON), high-fat diet (HF), or high-fat diet with CSE (HF+CSE) for an additional seven weeks. A 100 mg/kg of BW amount of CSE (dispersed in 50% milk in Tris-buffered saline containing 0.01% Tween 20 (TBS-T) for at least 1–2 h. The blocked membrane was then incubated overnight at 4°C with either phosphorylated-AMPK, phosphorylated-ACC, or β-actin. All the primary antibodies were purchased from Cell Signaling Technologies (Danvers, MA, USA). The membrane was then washed with TBS-T for 30 min and incubated with the secondary anti-rabbit IgG peroxidase-linked antibody (1:5000; Amersham, USA) in 2.5% skim milk/TBS-T for at least 1 h at room temperature. Specific bands were visualized with a chemiluminescence system (Bio-Rad, Hercules, CA, USA). The intensities of each band were quantified by using the ImageJ software (Bio-Rad).

Table 1. Contents of Pectolinarin and Pectolinarigenin in Cirsium setidens Extracts (CSE)

<table>
<thead>
<tr>
<th>CSE</th>
<th>Pectolinarin (mg/g)</th>
<th>Pectolinarigenin (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>46.19 ± 0.79</td>
<td>1.52 ± 0.03</td>
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</table>

Data are mean ± SD (n = 5).

**Cirsium setidens** Extract Inhibits Hepatic Steatosis

**Real-time PCR analyses.** Total RNA was extracted by using the Trizol™ reagent (Invitrogen, Carlsbad, CA, USA). Reverse transcription was carried out with 1 μg of total RNA by using the iScript™ cDNA synthesis kit (Bio-Rad) in the manufacturer’s manual. Real-time PCR was performed with a real-time PCR instrument, using an iQ™ SYBR green supermix kit (Bio-Rad). The following primer sequences were used for CPT1 ( carnitine palmitoyltransferase-1): forward 5'-GGA CTC TAC GCT TCT TCT AAT CGG-3' and reverse 5'-CTT AGA TCT CTC TCT CTT CAG GAA A-3'; for PPAR (peroxisome proliferator-activated receptor α): forward 5'-CGA AGA CAA AAA GGC AGC AGG-3' and reverse 5'-TGA TGG AAC TAC CAC ACC ACG CT-3'; for ACC1 (acyl-CoA carboxylase 1): forward 5'-GGG CAA ACT GGA GGT CAC ACC AGG-3' and reverse 5'-GCC TTT TTG TTT CCA GCA AA-3'; and for SREBP1c (sterol regulatory element binding transcription factor 1c): forward 5'-GGG GAT CTC TCT CTT AAC ATG GGA-3' and reverse 5'-GGT GGT GAT CAC TTG GCA-3'. The mRNA levels are expressed as the number-fold induction, relative to the GAPDH mRNA.

**Histological examination and hepatic lipid measurements.** Formalin-fixed and paraffin-embedded liver and epididymal adipose tissues were routinely processed for hematoxylin and eosin (H&E) staining. The liver histology was examined and graded according to the magnitude of steatosis, as described earlier. Briefly, the degree of hepatic steatosis was graded 1–4 based on the average percentage of fat-accumulated hepatocytes per field at 100× magnification under H&E staining (grade 1 = 6–16%, 2 = 6–33%, 3 = 33–66%, 4 = >66%).

**Lipids.** Lipids were obtained from the liver as described by Schwartz et al. for hepatic lipid extraction and measurement. Briefly, liver tissue (50 mg) was homogenized in 1 mL of PBS-10% EDTA (pH 7.4). The liver tissue homogenate was subsequently extracted with 2 mL of isopropanol-hexane-water (80:20:2), 0.5 mL of hexane-diethyl ether (1:1), and 1 mL of distilled water. The organic phase was collected, dried in N2 gas, and resuspended in ethanol. The TG and cholesterol concentrations in the hepatic lipid extract and in the plasma were enzymatically measured by using commercial kits (Bio-Clinical System, Gyeonggi-do, Korea) according to the manufacturer’s instructions.

**Animals and diets.** All animals were approved by the Institutional Animal Care and Use Committee at Kyung Hee University. Male C57BL/6 mice (4 weeks old, SLC, Japan) were housed in a room with controlled temperature (21–23°C), humidity (55–60%) and lighting (12-h light/dark cycle), and provided free access to a rodent diet and water. After acclimatization for 1 week, the animals were assigned by weight-matching to three groups. One group was fed a control diet (10% kcal from fat) purchased from Research Diets (New Brunswick, NJ, USA), and two groups were fed a high-fat diet (45% kcal from fat; Research Diets) for eight weeks to induce obesity. The mice then continued to be fed a control diet (CON), high-fat diet (HF), or high-fat diet with CSE (HF+CSE) for an additional seven weeks. A 100 mg/kg of BW amount of CSE (dispersed in 50% milk in Tris-buffered saline containing 0.01% Tween 20 (TBS-T) for at least 1–2 h. The blocked membrane was then incubated overnight at 4°C with either phosphorylated-AMPK, phosphorylated-ACC, or β-actin. All the primary antibodies were purchased from Cell Signaling Technologies (Danvers, MA, USA). The membrane was then washed with TBS-T for 30 min and incubated with the secondary anti-rabbit IgG peroxidase-linked antibody (1:5000; Amersham, USA) in 2.5% skim milk/TBS-T for at least 1 h at room temperature. Specific bands were visualized with a chemiluminescence system (Bio-Rad, Hercules, CA, USA). The intensities of each band were quantified by using the ImageJ software (Bio-Rad).
Results

CSE improved obesity induced by the high-fat diet

After 15 wk of feeding, the final body weights of the mice fed the high-fat diet (HF) were significantly higher than those of the mice fed the control diet (CON), indicating that the high-fat diet had induced obesity. The mice fed the high-fat diet with CSE (HF+CSE) showed a significantly lower body weight than the mice in the HF group (Table 2). CSE administration (100 mg/kg of BW) significantly suppressed the body weight gain by 21%, as compared to the HF group. There was no significant difference in the levels of daily food intake during the experimental period. The food efficiency ratio was significantly increased by HF feeding, but the CSE administration did not significantly change the food efficiency ratio in the HF+CSE group, as compared to the HF group.

A histological analysis of the epididymal adipose tissue confirmed that the adipocyte size was markedly larger in the HF group than in the CON group, whereas the adipocyte size was much smaller in the HF+CSE group than in the HF group (Fig. 1). In addition, the weight of the epididymal adipose tissue in the HF+CSE group was significantly lower by 32% than that in the HF group (p < 0.05). The weight of subcutaneous adipose tissue in the HF+CSE group was also significantly lower than that in the HF group (p < 0.05).

CSE attenuated hepatic steatosis and decreased the hepatic and plasma TG concentrations

The effect of CSE administration on the lipid accumulation in the liver of the mice fed the high-fat diet was examined by H&E staining. As expected, the mice fed the high-fat diet without CSE administration developed severe hepatic steatosis (Fig. 2). Similarly, the respective hepatic TG and cholesterol concentrations were 246% and 27% higher in the HF group than in the CON group.

CSE administration markedly improved hepatic steatosis as shown in Fig. 1A. The average grade of hepatic steatosis in the HF+CSE group was significantly lower than that in the HF group (3.67 ± 0.22 in HF vs. 2.25 ± 0.29 in HF+CSE, p < 0.05). CSE administration also significantly decreased the hepatic TG concentration (a 44% decrease, p < 0.05) and plasma TG concentration (a 27% decrease, p < 0.05) when compared to the HF group. CSE administration, however, had no effect on the hepatic and plasma cholesterol concentrations (Fig. 2, Table 2).

CSE induced the gene expression of fatty acid β-oxidation enzymes in the liver

To examine whether the CSE administration improved obesity and attenuated hepatic steatosis by modulating the expression of genes involved in lipid metabolism, the mRNA levels for CPT1, MCAD, ACC1, SREBP1c, PPARα, CPT2, and ACO were measured by a real-time PCR analysis.

The hepatic mRNAs for CPT1 and MCAD, the enzymes involved in fatty acid β-oxidation, were significantly decreased by HF when compared to the CON group (Fig. 3). On the other hand, the expression of these genes was significantly increased by CSE.

Table 2. Effects of Cirsium setidens Extracts (CSE) on Body Weight Gain, Food Intake, Food Efficiency Ratio, Liver Weight, and Plasma TG and Cholesterol Concentrations in the High-Fat Diet-Induced Obese Mice

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>HF</th>
<th>HF+CSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight (g)</td>
<td>18.1 ± 0.2</td>
<td>18.5 ± 0.2</td>
<td>18.3 ± 0.2</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>24.0 ± 0.5a</td>
<td>33.3 ± 1.4a</td>
<td>29.6 ± 1.4b</td>
</tr>
<tr>
<td>Body weight gain (g)</td>
<td>5.8 ± 0.6a</td>
<td>14.5 ± 1.3b</td>
<td>11.4 ± 1.3b</td>
</tr>
<tr>
<td>Food intake (g/d)</td>
<td>2.5 ± 0.0</td>
<td>2.4 ± 0.1</td>
<td>2.4 ± 0.1</td>
</tr>
<tr>
<td>Food efficiency ratio</td>
<td>0.02 ± 0.002a</td>
<td>0.06 ± 0.004b</td>
<td>0.05 ± 0.004b</td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>1.0 ± 0.1a</td>
<td>1.3 ± 0.1b</td>
<td>1.1 ± 0.0ab</td>
</tr>
<tr>
<td>Plasma TG (mg/dL)</td>
<td>68.4 ± 4.9b</td>
<td>63.1 ± 3.1b</td>
<td>45.9 ± 5.0b</td>
</tr>
<tr>
<td>Plasma cholesterol (mg/dL)</td>
<td>105.0 ± 7.4a</td>
<td>133.0 ± 9.0b</td>
<td>141.1 ± 4.9b</td>
</tr>
</tbody>
</table>

Data are mean ± SE (n = 10). Different letters indicate significant difference (p < 0.05).
administration in the liver tissues of the high-fat diet fed mice by about 1.8-fold (CPT1) and 1.7-fold (MCAD) of the levels in the HF group ($p < 0.05$) (Fig. 3). The mRNA level of PPARα was significantly lower in the HF group than in the CON group. The PPARα mRNA level tended to be higher in the HF+CSE group than in the HF group, although the difference was not statistically significant (Fig. 3). The mRNA levels of CPT2 and ACO were no different among the three groups.

No difference was found in the hepatic mRNA levels for ACC1 and SREBP1c, both being involved in lipogenesis, between the liver of the mice fed the high-fat diet with or without CSE administration (Fig. 3).

**Effects of CSE on the phosphorylation of AMPK and ACC in the mice fed the high-fat diet**

We also examined the protein levels of phosphorylated-AMPK and phosphorylated-ACC in the liver tissue of mice from the three groups. As shown in Fig. 4, the level of phosphorylated-AMPK was significantly lower in the HF group to about 47% of that in the CON group. The phosphorylation of AMPK in the HF+CSE group was restored to a level similar to that in the CON group. The level of phosphorylated-ACC, however, was not significantly different among the three groups.

**Discussion**

In accordance with previous studies, the body weight and visceral adipose tissue weight were significantly higher in the mice fed a high-fat (45% kcal from
fat) diet than in the mice fed a low-fat (10 kcal from fat) control diet. The mice in the HF group also showed a large number of lipid droplets and increased TG and cholesterol concentrations in the liver tissue, confirming the development of hepatic steatosis in this animal model. On the other hand, CSE administration to the mice fed the high-fat diet significantly decreased the final body weight as well as the epidymidal and subcutaneous adipose tissue weights. Furthermore, CSE administration markedly decreased the number of lipid droplets in the liver tissue as well as significantly decreasing the hepatic and plasma TG concentrations. The daily food intake, however, did not differ among the three groups. These results clearly show that CSE effectively improved the hepatic steatosis induced by the high-fat diet, without affecting the amount of food ingested. Previous studies have shown that *C. setidens* or its component, pectolinarin, protected against liver damage induced by either N-galatosamine or CCl4, mostly via its antioxidative activity, although whether or not it could modulate hepatic lipid metabolism had not been studied. Our study results therefore provide a novel role for *C. setidens* as an effective anti-obese and hepatoprotective agent in vivo.

Although we did not determine the serum concentration of CSE components after the intervention, previous studies have shown that the oral administration of a *C. setidens* extract at a dose (100 mg/kg BW) similar to that used in our study elicited various biological activities in in vivo models, suggesting that the oral administration of a *C. setidens* extract at this dose could effectively raise the serum and/or tissue levels of the *C. setidens* extract components. The study by Yoo et al. has also shown that the oral gavage of a *C. setidens* extract (at a dose of 100 mg/kg of BW) for just two weeks prevented galactosamine-induced liver damage in mice. In our study, the intervention period was 7 weeks, which is more than three times longer than the one used in the study by Yoo et al. It is therefore likely that the treatment with CSE at a dose of 100 mg/kg of BW for 7 weeks was sufficient to raise the body pool of CSE components.

We examined the expression levels of the genes related to lipid metabolism in the liver to elucidate the mechanism for the action of CSE in preventing hepatic fat accumulation. We found that CSE strongly induced the gene expression of CPT1 and MCAD by about 2-fold when compared to the HF group without CSE. The CPT1 and MCAD genes encode the key enzymes involved in fatty acid β-oxidation, and both of these genes were significantly down-regulated in the HF group when compared to the CON group. The expression of such other lipolytic genes as CPT2 and ACO was different between the groups. CPT1 mediates the transport of long-chain fatty acids into the mitochondria for β-oxidation and therefore is the rate-limiting enzyme in mitochondrial fatty oxidation. MCAD also catalyzes the initial reaction of fatty acid oxidation in the mitochondria. The substrates for MCAD include medium-chain length acyl-CoA thioesters derived from medium-chain fatty acids that enter the mitochondria, products of the mitochondrial oxidation of long-chain fatty acids, and products of the peroxisomal oxidation of long-chain fatty acids. Since these diverse pathways for fatty acid oxidation converge at this point, MCAD catalyzes a pivotal step in cellular fatty acid metabolism. It has been shown that the hepatic MCAD mRNA level was in parallel to the β-oxidation activity in the liver, and that the body weight increase induced by high-fat feeding in mice was related to lower fatty acid β-oxidation activity in the liver, suggesting hepatic MCAD gene expression would play an important role in regulating lipid and energy metabolism. Similar to our observation, Ohnogi et al. have shown that hypertriglyceridemia in rats fed a high-fructose drink was associated with the suppression of CPT1 and MCAD gene expression in the liver tissue, and the enhanced expression of MCAD by an *Angelica keiskei* extract normalized the TG concentration to the control level. Dietary genistein supplementation (0.2% and 0.4%) also dose-dependently decreased the hepatic TG concentration in mice fed the high-fat diet via an increase in the MCAD mRNA level in the liver. These results indicate that the up-regulation of fatty acid β-oxidation gene expression such as CPT1 and MCAD was critical in suppressing fat accumulation in the liver tissue. It is therefore likely that the induction of CPT1 and MCAD gene expression by CSE mediated the prevention of hepatic steatosis in mice fed the high-fat diet in this study.

Although the mechanism by which CSE stimulates the expression of MCAD is unknown, the gene expression of MCAD is known to be regulated by PPARα (peroxisome proliferator-activated receptors). We have shown in our study that the hepatic mRNA level for PPARα was significantly down-regulated in the HF group when compared to the CON group. Furthermore, hepatic PPARα mRNA tended to be higher in the HF+CSE group than in the HF group, although the difference was not statistically significant. It is possible that the induction of MCAD and CPT1 by CSE, which promotes fatty acid β-oxidation, is associated with PPARα activation. In support of this concept, treating with the PPARα agonist finofibrate has been shown to induce the mRNA expression of fatty acid oxidation enzymes including MCAD and CPT, and to improve hepatic steatosis in OLETF rats, the animal model of NAFLD.

AMPK is known to play a major role in lipid metabolism. Once activated, AMPK phosphorylates and inactivates a number of metabolic enzymes including ACC. Inhibiting ACC leads to a drop in malonyl-CoA which is both a critical precursor for the biosynthesis of fatty acids and a potent inhibitor of mitochondrial fatty acid oxidation. In addition, AMPK activation has been shown to decrease the expression of such lipogenic enzymes as FAS (fatty acid synthase). We found that the protein level of phosphorylated-AMPK was significantly increased by CSE. However, the level of phosphorylated ACC, the downstream target of activated AMPK, was not significantly changed by CSE in our study. Consistent with these results, the hepatic FAS mRNA level was not significantly different between mice fed the high-fat diet with or without CSE (data now shown). These data indicate that the inhibitory effect of CSE against fat accumulation observed in our study is likely to have been due to increased lipid breakdown rather than to decreased lipid biosynthesis.
It should be noted that *C. setidens* contains various biologically active flavonoids, including pectolinarin, luteolin, and apigenin. Pectolinarin, one of the most abundant flavonoids found in *C. setidens*, has been shown to have an anti-diabetic effect on diabetic rats established by injecting streptozotocin and subsequent feeding with a high-carbohydrate/high-fat diet. A recent study has reported that pectolinarin modulated adipocyte differentiation by inducing PPARγ activation. On the other hand, luteolin had a lipid-lowering effect in feeding with a high-carbohydrate/high-fat diet.

Recent studies are warranted to further characterize the component(s) of the *C. setidens* extract that have a protective effect against nonalcoholic hepatic steatosis.

In conclusion, this study has clearly demonstrated that CSE protected against the development of hepatic steatosis induced by a high-fat diet in mice by activating AMPK activation or PPARγ regulation. Future studies are warranted to further characterize the component(s) of the *C. setidens* extract that have a protective effect against nonalcoholic hepatic steatosis.

**Acknowledgments**

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**References**