Ethnopharmacological communication

Anti-obesity effect of *Morus bombycis* root extract: Anti-lipase activity and lipolytic effect

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\textbf{A B S T R A C T}

\textbf{Aim of the study:} This study evaluated anti-obesity effect of the ethanolic extract of *Morus bombycis* root on lipase activity and lipolysis in adipocytes and adipose tissues.

\textbf{Materials and methods:} Lipase (triacylglycerol acylhydrolase, EC 3.1.1.3) activity was determined by measuring the hydrolysis of p-nitrophenyl butyrate to p-nitrophenol at 405 nm. Lipolytic effects were assayed in fully differentiated 3T3-L1 adipocytes and adipose tissues. In vitro, phosphodiesterase (PDE) activity was also measured.

\textbf{Results:} *Morus bombycis* root extract exhibited strong anti-lipase activity, with an IC\textsubscript{50} value of 2.07 \(\mu\)g/mL.

In differentiated adipocytes and adipose tissues, the extract increased lipolytic effects such as decreased intracellular triglyceride and the release of glycerol. Further, the extract inhibited PDE activity in a dose-dependent manner.

\textbf{Conclusion:} The present study suggests that *Morus bombycis* root extract might be of therapeutic interest with respect to the treatment of obesity.

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1. Introduction

*Morus bombycis* Koidzumi (Moraceae), is widely distributed in Asia and used in Korean traditional medicine to treat metabolic diseases, such as diabetic mellitus and hyperlipemia (Jin et al., 2007). The extracts and constitutes of *Morus bombycis* root have been reported to possess several pharmacological properties such as hepatoprotective activity, antioxidant effects, inhibitory effects of protein tyrosine phosphatase 1B, and reducing left ventricular developed pressure and heart rate in spontaneously hypertensive rats (Heo et al., 2007; Hoang et al., 2009). Asano et al. (1994) also observed the leave and root of *Morus bombycis* contain many polyhydroxylated alkaloids which are potent and highly selective glycosidase inhibitors. Up until now, no reports describing the anti-obesity effect of this plant have been published. This paper reports that the ethanolic extract of *Morus bombycis* root shows the anti-lipase activity by pancreatic lipase assay and lipolytic effects in 3T3-L1 adipocytes and adipose tissues.

2. Materials and methods

2.1. Materials

*Morus bombycis* Koidzumi was collected in South Korea and it has been authenticated by Professor J. H. Kim, Division of Life Science, Kyungwon University, Seongnam, Korea. A voucher specimen (KIOI-TBRC-K6-21) has been deposited at the Herbarium of Diabetic Complications Research Center, Korea Institute of Oriental Medicine.

Lipase (Type II; from Porcine pancreas), orlistat, and p-nitrophenyl butyrate were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All reagents were of biochemical grade.

2.2. Preparation of plant extracts

The dried and powdered plant (200 g) was extracted three times with 99.8% EtoH, and extracts were obtained by removal of the solvent in vacuo. Concentrated samples were stored at \(-20^\circ\) C for further studies. The percentage yield of the ethanolic extracts was found to be 3.1% (w/w). Extracts were dissolved in DMF at a concentration that in the total volume (3%) did not affect enzyme activity.
2.3. Measurement of pancreatic lipase activity

The method for measuring pancreatic lipase activity was modified from that of Kim et al. (2009). Briefly, an enzyme buffer was prepared by the addition of a solution of porcine pancreatic lipase [2.5 mg/mL in 10 mM MOPS (morpholinepropanesulfonic acid) and 1 mM EDTA, pH 6.8] to 169 L of the enzyme buffer and incubated for 15 min at 37 °C with 5 mM CaCl2, pH 7.0. Then, either 20 L of the compound at the test concentration, or orlistat, was mixed with 20 L of the enzyme buffer and incubated for 15 min at 37 °C with 5 mM CaCl2, pH 7.0. The enzymatic reactions were allowed to proceed for 30 min at 37 °C. Lipase activity was determined by measuring the hydrolysis of p-NPB to p-nitrophenol at 405 nm using an ELISA reader (BIO-TEK, Synergy HT, VT, USA). Inhibition of lipase activity was expressed as the percentage decrease in OD when porcine pancreatic lipase was incubated with the test compounds. Lipase inhibition (%) was calculated according the following formula:

\[ \text{Inhibition} \% = 100 - \left( \frac{B - b}{A - a} \times 100 \right) \]

where \( A \) is the activity without inhibitor, \( a \) is the negative control without inhibitor, \( B \) is the activity with inhibitor, and \( b \) is the negative control with inhibitor.

2.4. Culture and differentiation

3T3-L1 preadipocytes (ATCC, Rockville, MD, USA) were cultured in 4.5 g/L glucose-DMEM with 10% calf serum, penicillin (100 U/mL), streptomycin (100 g/mL), and streptomycin (100 U/mL) in 10-cm plastic Petri dishes until they reached 100% confluence. For differentiation, 2-day post-confluent cells were incubated for 48 h in DMEM with 10% FBS, antibiotics, and a differentiation cocktail termed MDI, which contained 0.5 mM isobutylmethylxanthine, 1 mM dexamethasone, 1 mM IBMX, and 1 g/mL insulin. Cells were cultured for 14 days in DMEM with 10% FBS and antibiotics, and the media changed every 2 days until the cells were collected for analysis.

2.5. Oil red O staining for intracellular triglycerides

Cells were fixed on dishes with 3% formaldehyde in PBS for 20 min. After two rinses with PBS, cells were incubated with an Oil red O solution (0.5% Oil red O, 60% isopropanol, and 40% water) for 30 min. The monolayer was extensively washed with water to remove unbound dye. Representative images of treated cells were obtained with an Olympus microscope (BX51, Japan), equipped with an Olympus DP 70 camera. Stained cells were air-dried overnight and then dissolved in isopropanol. Absorbance was measured at 520 nm.

2.6. Determination of glycerol release from rat adipose tissue

Glycerol release was used to assess the lipolytic effect from rat adipose tissue and examined according to a previous study (Kim et al., 2009). Briefly, epididymal adipose from male Wister rats (6 ss old, Orient Co., Kyungki-do, Korea) were removed under light ether anesthesia. Fat cells were isolated from the adipose tissue using collagenase (10 mg/1 g of tissue) digestion by the method of Rodbell (1964) and treated with 200 L of Hanks balanced solution supplemented with 2.5% bovine serum albumin (BSA). Fat cells were incubated with 0.8 mL glycerol reagent and 25 mL of isoproterenol (final concentration, 2.5 g/mL) or the extracts of Morus bombycis (final concentration, 1 and 2.5 g/mL) for 1 h at 37 °C. Released glycerol was assayed using free glycerol regent from Sigma Chemical Co. (Cat. F6428). The absorbance of the solution was measured at a wave length of 540 nm using a microplate reader (Synergy HT, BioTek) and calculated according the following formula:

\[ \text{Glycerol} = \frac{\text{abs. of sample} - \text{abs. of blank}}{\text{abs. of standard} - \text{abs. of blank}} \times \text{concentration of standard.} \]

2.7. Phosphodiesterase (PDE) activity assay

PDE activities were assayed using the PDE-GloTM phosphodiesterase activity kit (Promega Corp., WI, USA) according to the manufacturer’s instructions. Briefly, PDE enzyme and extract were incubated with cAMP (1 mM) to initiate the PDE reaction. The PDE-GloTM termination buffer containing the PDE inhibitor and detection solution were added and mixed well. The Kinase-Glo reagent was added and incubated for 10 min at room temperature. Luminescence was measured using a plate-reading luminometer (BIO-TEK, Synergy HT, VT, USA).

2.8. Statistical analysis

Data were expressed as the mean ± standard error mean or deviation (PRISM software, Graph Pad, San Diego, CA, USA). A difference in the mean P values of P < 0.05 was considered as significant.

3. Results and discussion

Obesity results from the disequilibrium between energy intake and expenditure. It is believed to be associated with numerous diseases, including hyperlipidemia, hypercholesterolemia, and type 2 diabetes. Recently, newer approaches for the treatment of obesity have involved inhibition of dietary triglyceride absorption via inhibition of pancreatic lipase as this is the major source of excess calories (Birari and Bhutani, 2007). Phytochemicals identified from traditional medicinal plants present an exciting opportunity for

### Table 1

<table>
<thead>
<tr>
<th>Scientific name</th>
<th>Family name</th>
<th>Part used</th>
<th>Concentration (µg/mL)</th>
<th>Inhibition (%)</th>
<th>IC50 (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morus bombycis</td>
<td>Moraceae</td>
<td>Root</td>
<td>1.5</td>
<td>47.07 ± 1.77</td>
<td>2.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>48.41 ± 1.63</td>
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<td></td>
<td></td>
<td></td>
<td>2.5</td>
<td>53.18 ± 0.65</td>
<td>1.06</td>
</tr>
<tr>
<td>Morus bombycis</td>
<td>Moraceae</td>
<td>Stem</td>
<td>20</td>
<td>45.72 ± 4.07</td>
<td>28.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>35</td>
<td>54.28 ± 3.91</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>50</td>
<td>59.67 ± 1.21</td>
<td>94.08</td>
</tr>
<tr>
<td>Morus bombycis</td>
<td>Moraceae</td>
<td>Twig, stem, leaf</td>
<td>100</td>
<td>44.33 ± 2.44</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>Orlistat (positive control)</td>
<td>Moraceae</td>
<td>Twig, stem, leaf</td>
<td>0.025</td>
<td>18.03 ± 2.98</td>
<td>0.076 (0.154 µM)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.05</td>
<td>35.64 ± 1.25</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>0.1</td>
<td>63.08 ± 1.41</td>
<td></td>
</tr>
</tbody>
</table>

a Results are the mean ± SD (n = 4).
the development of newer therapeutics. As part of the continuing search for biologically active anti-obesity agents from natural herbal resources, various plants have been screened for their anti-lipase activity (Birari and Bhutani, 2007).

Pancreatic lipase inhibition of Morus bombycis extracts is expressed as percentage (%) and IC50 value (the concentration required to inhibit a lipase activity by 50%) (Table 1). Morus bombycis root extract exhibited the strongest inhibitory effect on lipase with an IC50 value of 2.07 μg/mL. However, it was not more effective than orlistat (IC50 = 0.076 μg/mL (0.154 μM)), a positive control. Orlistat has serious side effects, such as gas with oily spotting, stomach pain, irregular menstrual periods, and headaches.

Fig. 1. Oil red staining of the Morus bombycis root extract in 3T3-L1 adipocytes. (A) Oil red O staining for intracellular triglycerides in 3T3-L1 adipocytes. 3T3-L1 adipocytes were induced with Morus bombycis (0–100 μg/mL) for 72 h. (B) Relative density of Oil red O staining. Data are expressed as the mean ± S.E.M. (n = 3). **P<0.01, ***P<0.001 vs. untreated cells, respectively.
showed that they accumulated substantial amounts of cytoplasmic triglycerides, which are contained inside lipid droplets. In differentiated 3T3-L1 adipocytes, lipid droplets in untreated control cells stained very strongly with Oil red O, an indication that the cells accumulated substantial amounts of cytoplasmic triglycerides. Representative images of Oil red O staining in treated cells showed that Morus bombycis root extract suppressed intracellular triglyceride accumulation in a dose-dependent manner (Fig. 1A and B). To determine the lipolytic effect of the Morus bombycis root extract in adipose tissues, glycerol release was used as an indicator of lipolysis as described in Materials and methods. Isoproterenol stimulates lipolysis via beta-adrenergic receptor activation and cAMP-dependent signaling (Robidoux et al., 2006). Glycerol release was increased by Morus bombycis root extract in a dose-dependent manner (Fig. 2A). PDE inhibitors (PDEIs) are a class of drugs that are widely used because of their various pharmacological properties; inhibition of NF-kappaB activation and the subsequent induction of proinflammatory mediators and absence of effect of food on absorption. Thus they may have potential as therapeutic targets for inflammatory disease and obesity (Rahimi et al., 2010; Sato et al., 2006). Fig. 2B indicates that the Morus bombycis root extract has an inhibitory effect on PDE activity in vitro assay. This study has first clarified that the Morus bombycis root extract acts as a potent anti-obesity by means of intracellular accumulation of cAMP through the inhibition of PDE. Further studies of Morus bombycis on toxicity are necessary to evaluate its mechanism of action and to fully establish its safety profile.

4. Conclusions

Our results reveal, for the first time, that Morus bombycis root extract has a strong anti-lipase activity and PDE inhibition in vitro. The extract shows lipolytic effect in adipocytes and adipose tissues. Thus, it is worthwhile to further investigate this extract for its potential pharmacological effect in metabolic disorders, specifically obesity.

Acknowledgements

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References


