The methanol extract of *Dypsis lutescens* leaves showed inhibitory effects on lipase activity *in vitro* and on triglyceride accumulation in 3T3-L1 pre-adipocytes. Further experiments using the extract on mice demonstrated a suppressive effect on the postprandial elevation of blood triglyceride level and an anti-obesity effect on obese mice induced by a high-fat diet. *D. lutescens* will accordingly be useful for preventing obesity.

**Key words:** *Dypsis lutescens*; anti-obesity effect; lipase inhibitor; 3T3-L1; high-fat diet

Obesity, an abnormal condition of accumulating excessive triglyceride in adipose tissue, is the most important risk factor of such symptoms of the metabolic syndrome as hypertension, type II diabetes and hyperlipemia.1,2 The metabolic syndrome also develops atherosclerotic diseases, whose fatality is very high, when the symptoms gets worse.3,4 Preventing and reducing obesity is known to be important to maintain health, and functional food materials can help to achieve this objective.

Our preliminary screening of various plant extracts identified inhibitory activities against pancreatic lipase *in vitro* and against triglyceride accumulation during the differentiation of 3T3-L1 pre-adipocytes in a methanol extract of *Dypsis lutescens* (MeDL) leaves. We describe here the details and potential value of the extract as a new material for functional foods.

*D. lutescens*, which is commonly known as the golden cane palm or areca palm, is widely distributed as a foliage plant in the tropics and sub-tropics. Although the leaf is used to satisfy a daily need in these regions, its biological activities have not previously been reported. The prospective anti-obesity action of the palm leaf prompted us to examine the biological activities of an extract by *in vivo* experiments. Fresh leaves of the *D. lutescens* palm, which had been obtained from Akatsuka Botanical Garden Co. (Mie, Japan), were removed from the leaf stem and cut into small pieces to extract twice with methanol (10 L each) for 2 weeks. The extract solution was filtered and evaporated to give a dark green-colored powder, the yield of the powder from the fresh leaves being 10.5% (w/w). The powder was kept in a freezer until needed, the extract being resolved or mixed with vehicles for subsequent experiments as the methanol extract of *D. lutescens* leaves (MeDL).

One of the most effective ways to inhibit the absorption of triglyceride is thought to be the inhibition of lipase. The inhibitory effect of MeDL on lipase activity *in vitro* was measured by using porcine pancreatic lipase (ICN Biomedicals, Ohio, USA) and Lipase Kit S (DS Pharma Biomedicals, Osaka, Japan) according to the manufacturer’s protocol with minor modifications for evaluating inhibitory activity.5) The lipase activity (%) was calculated by defining the intact enzyme reaction as 100%. Data are expressed as the mean ± SE, and the statistical significance was evaluated by ANOVA and a subsequent Tukey post-hoc analysis, where *p* < 0.05 was considered to be statistically significant. Figure 1A shows that the MeDL treatment (250–1,000 μg/mL) significantly suppressed the lipase activity when compared with the control.

To confirm the onset of action *in vivo*, the effect of MeDL on the triglyceride (TG) absorption was investigated by a corn oil-loading test on 7-week-old male Slc:ddY mice. All animal studies were conducted according to the 2006 guidelines in Notification no. 88 of Ministry of the Environment in Japan and Guidelines for Animal Experimentation of Tokyo University of Marine Science and Technology with the approval of the Animal Care and Use Committee of Tokyo University of Marine Science and Technology. After being fasted for 24 h, the mice were assigned to 4 groups. MeDL (500, 1,000, or 1,500 mg/20 mL/kg) or distilled water (DW) as the control was orally administered to the respective group before an oral administration of corn oil (8 mL/kg) was given. The area under curve (AUC) value was calculated from a time-course plot of plasma TG as the index of the total absorbed amount. The elevation of plasma TG level in the 500, 1,000 and 1,500 mg/kg MeDL groups was significantly lower than that in the control group (Fig. 1B). The evaluation by AUC value showed the figure for the MeDL groups (1,000 mg/kg, 3386 ± 407 mg·h/dL; 1,500 mg/kg, 2813 ± 287 mg·h/dL) to be significantly lower than that of the control group (5701 ± 879 mg·h/dL). These results suggest that MeDL depressed TG absorption from the small intestine by inhibiting the pancreatic lipase activity.
Other in vitro experiments were then performed with adipocytes. The suppressive effect of MeDL on the TG accumulation in adipocytes was evaluated by using cells from the 3T3-L1 murine white pre-adipocyte line during differentiation. 3T3-L1 pre-adipocytes provided by Health Science Research Resources Bank were cultured in Dulbecco’s modified Eagle’s medium (DMEM, Invitrogen, Osaka, Japan) supplemented with 10% calf serum (Sanko Junyaku, Tokyo, Japan). Two days after confluence, differentiation was induced for 2 d by DMEM containing 10% fetal bovine serum (FBS, Tissue Culture Biologicals, CA, USA), 0.5 mM of 3-isobutyl-1-methylxanthine, 0.25 µM of dexamethasone, and 10 µg/mL of insulin (each from Sigma-Aldrich, MO, USA), and MeDL (0–50 µg/mL). The medium was replaced every second day for 12 d with DMEM containing 10% FBS, 5 µg/mL of insulin, and MeDL (0–50 µg/mL). The TG accumulation and cell viability were respectively evaluated according to the methods of Saito et al.,60 and Chien et al.,7 MeDL (12.5–50 µg/mL) significantly decreased the rate of TG accumulation in 3T3-L1 cells when compared to the control without MeDL (Fig. 2A). There was also no difference in the cell viability between the control and MeDL (3.125–50 µg/mL). The inhibitory effect of MeDL on the activity of glycerol-3-phosphate dehydrogenase (GPDH), a biomarker of adipogenesis, was next evaluated by using a GPDH Activity Measurement kit (Primary Cell, Hokkaido, Japan) according to the manufacturer’s protocol. MeDL (3.125–50 µg/mL) significantly inhibited the GPDH activity in 3T3-L1 cells when compared to the control (Fig. 2B). These results indicate that MeDL suppressed the TG accumulation by inhibiting GPDH activity in the adipocytes. It has been reported that GPDH expression was regulated by peroxisome proliferator-activated receptor γ (PPARγ),80 and that the expression of GPDH and PPARγ was also controlled by insulin in white adipocytes.9,10 It would therefore be interesting to elucidate in a future study whether MeDL inhibited the GPDH activity by depressing the insulin signal or PPARγ expression.

Based on the foregoing results, we evaluated the anti-obesity effect of MeDL in a long-term experiment for 11 weeks on obese mice induced by a high-fat diet (HFD). Female C57Bl/6J mice (3 weeks old) were preliminarily fed with a normal diet (ND) for 1 week and then assigned to three groups: the ND group, HFD group, and 3% MeDL group. ND contained 4% beef tallow, 14% casein, 62.07% cornstarch, 10% sucrose, 5% cellulose, 3.5% mineral mixture, 1% vitamin mixture, 0.18% l-cystine, 0.25% choline bitartrate, and 0.0008% tertbutylhydroquinone. HFD contained 40% beef tallow,
Table 1. Effects of MeDL on High-Fat Diet-Fed Mice for 11 Weeks

<table>
<thead>
<tr>
<th>Group</th>
<th>ND</th>
<th>HFD</th>
<th>3% MeDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>31.6 ± 1.2**</td>
<td>45.8 ± 3.3</td>
<td>37.5 ± 1.7**</td>
</tr>
<tr>
<td>Adipose tissue (mg/g of BW)</td>
<td>28.0 ± 4.5**</td>
<td>98.0 ± 14.3</td>
<td>47.6 ± 7.4**</td>
</tr>
<tr>
<td>Liver (mg/g of BW)</td>
<td>36.4 ± 1.8</td>
<td>31.8 ± 1.3</td>
<td>33.9 ± 1.2</td>
</tr>
<tr>
<td>Hepatic TG (mg/g of liver)</td>
<td>19.0 ± 1.1**</td>
<td>32.0 ± 1.1</td>
<td>25.4 ± 1.3**</td>
</tr>
<tr>
<td>Hepatic TC (mg/g of liver)</td>
<td>6.96 ± 0.21*</td>
<td>9.01 ± 0.78</td>
<td>6.98 ± 0.29*</td>
</tr>
<tr>
<td>Plasma TG (mg/dL)</td>
<td>87.2 ± 5.7*</td>
<td>117.7 ± 8.2</td>
<td>104.9 ± 7.6</td>
</tr>
<tr>
<td>Plasma TC (mg/dL)</td>
<td>144.1 ± 6.2*</td>
<td>185.2 ± 17.1</td>
<td>134.0 ± 3.4*</td>
</tr>
<tr>
<td>Plasma NEFA (mEq/L)</td>
<td>1.47 ± 0.04**</td>
<td>1.66 ± 0.03</td>
<td>1.41 ± 0.02**</td>
</tr>
<tr>
<td>Plasma glucose (mg/dL)</td>
<td>78.1 ± 3.9*</td>
<td>93.5 ± 5.1</td>
<td>75.6 ± 2.5*</td>
</tr>
<tr>
<td>Feces (mg/d)</td>
<td>207.0 ± 6.4*</td>
<td>230.9 ± 4.2</td>
<td>240.6 ± 3.9</td>
</tr>
<tr>
<td>Fecal TG (mg/d)</td>
<td>0.44 ± 0.21</td>
<td>1.37 ± 0.15</td>
<td>2.39 ± 0.35*</td>
</tr>
<tr>
<td>Fecal TC (mg/d)</td>
<td>4.32 ± 0.27**</td>
<td>6.75 ± 0.69</td>
<td>8.78 ± 0.20*</td>
</tr>
</tbody>
</table>

ND, normal diet; HFD, high-fat diet; 3% MeDL, HFD containing 3% methanol extract of Dypsis lutescens leaves. Data are presented as the mean ± SE and analyzed by ANOVA followed by a Tukey post-hoc analysis. *p < 0.05, **p < 0.01 vs. HFD.

Suppressed Fat Accumulation in Mice by a Palm Leaf Extract

14% casein, 26.07% cornstarch, 10% sucrose, 5% cellulose, 3.5% mineral mixture, 1% vitamin mixture, 0.18% L-cystine, 0.25% choline bitartrate, and 0.0008% tert-butylhydroquinone. The 3% MeDL group were fed on HFD containing 3% MeDL. The feces of each group were collected from the 79th to 83rd day after feeding started. The hepatic and fecal lipids were extracted according to the method of Folch et al.11) There was hardly any difference in the food intake for 11 weeks between the HFD (213.8 g/mouse) and 3% MeDL (214.1 g/mouse) groups. Table 1 shows that the body weight on the 77th day after feeding started, the para-uterus adipose tissue weight, and the hepatic TG, plasma TC and glucose levels in the 3% MeDL group were significantly lower than those in the HFD group. These results demonstrate that MeDL depressed the visceral fat accumulation caused by HFD and the elevation of the plasma TG and glucose levels induced by obesity. Moreover, the fecal TG and TC levels in the 3% MeDL group were significantly higher than those in the HFD group. The inhibitory activities of MeDL against adipogenesis in adipocytes have a tenuous connection with the anti-obesity activity. However, the enhanced cholesterol excretion in the feces and its resulting hepatic and plasma cholesterol-lowering effects (Table 1) suggest that both the facilitation of TG excretion in the feces and the inhibition of adipogenesis in white adipocytes contributed to the anti-obesity action of MeDL. Details of the mechanism of MeDL for its anti-obesity effects are a key topic for future research.

The suppressive effect on benign prostate enlargement of saw palmetto berry,12) the antioxidative and anti-inflammatory activities of the polyphenol-rich fraction of acai berry,13) and the suppressive effect on liver damage of the fruit extract of Phenix sp.14) have been reported. However, there has been no report on the bioactivities of a palm leaf extract. We confirmed the inhibitory activity of the MeOH extract of several palm leaves (50 μg/mL without cytotoxicity) by in vitro tests. TG accumulation in adipocytes was suppressed to 69.5 ± 2.7% by Satakentia liukiunensis (Yaeayama-yashi), 65.9 ± 4.6% by Trachycarpus fortunei (Shuro), 105.2 ± 6.2% by Mascarena verschaffeltii (Tokkuri-yashimodoki), and 95.2 ± 8.8% by Chamaerops humilis (Chabo-tou-juro) when compared to that of control values (the Japanese name has been given in parentheses). MeDL showed the most prominent effects against TG accumulation (53.7 ± 4.8%) among palm leaf extracts we tested. It has been reported that palm oil contained abundant unsaturated fatty acids, phytosterols, and such antioxidants as carotenoids and tocotrienols.15) In particular, tocotrienols, one of the most effective antioxidants in vivo, have demonstrated nephroprotective activity in type 1 diabetic rats,16) apoptosis-inducing activity against a breast cancer cell line,17) and suppressive activity against TG accumulation and differentiation in adipocytes 3T3-L1.18) However, these compounds in palm oil have not so far been found as characteristic contents in the palm leaf extract. MeDL was separated by solvent partition in our preliminary experiments to give an ethyl acetate-soluble layer (38.7%, w/w) and water-soluble layer (61.3%, w/w). The inhibitory activity against lipase was shown in only the former layer, so at least the lipase inhibitor in MeDL was considered to be a lipophilic compound. Several compounds may be associated with the activities found in MeDL, and further experiments are needed to elucidate the mechanisms and active compounds involved.

In conclusion, MeDL demonstrated inhibitory effects on TG absorption in mice, on pancreatic lipase activity, and on TG accumulation in adipocytes by inhibiting GPDH activity. It is considered that MeDL demonstrated an anti-obesity effect based on these inhibitory actions. The leaf of Dypsis lutescens is therefore likely to become effective for preventing and reducing the metabolic syndrome, and particularly obesity.

References


