Hypolipidemic and hypoglycemic effects of *Centella asiatica* (L.) extract  

*in vitro* and *in vivo*

Nattapon Supkamonseni¹, Aree Thinkratok¹, Duangdeun Meksuriyen² & Rungrudee Srisawat¹*  
¹Institute of Science, Suranaree University of Technology, 111 University Avenue, Suranaree District, Amphur Muang, Nakhon Ratchasima 30000, Thailand  
²Faculty of Pharmaceutical Sciences, Chulalongkorn University, 254 Phyathai Road, Pathumwan, Bangkok 10330, Thailand  

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*In vitro* study revealed that pancreatic lipase inhibitory activity of *C. asiatica* extract was significantly higher than rutin but lower than orlistat, an anti-obesity drug. α-Amylase inhibitory activities of *C. asiatica* extract and rutin were significantly lower than acarbose, an anti-diabetic drug. Inhibition of α-glucosidase activity by *C. asiatica* extract, rutin, and acarbose was not different. The *in vivo* study substantiated the *in vitro* results. *C. asiatica* extract (1000 and 2000 mg/4 mL/kg), rutin (1000 mg/4 mL/kg), and orlistat (45 mg/4 mL/kg) significantly decreased plasma glucose, triglyceride and total cholesterol levels in lipid emulsion-induced hyperlipidemic rats at 3 h. However, plasma aspartate aminotransferase and alanine aminotransferase levels did not show significant change. The present work further supports that the *C. asiatica* extract and its bioactive rutin may help managing hypolipidemic and hypoglycemic effects.

**Keywords:** Acarbose, *Centella asiatica*, Hypoglycemia, Hypolipidemia, Lipid emulsion-fed rat, Obesity, Orlistat, Rutin

Obesity has been implicated in the development of hyperlipidemia, hyperglycemia, and hypertension¹. Important strategies for prevention of hyperlipidemia and hyperglycemia include inhibition of fat digestive enzymes (e.g. pancreatic lipase and pancreatic cholesterol esterase) and carbohydrate digestive enzymes (e.g. α-amylase and α-glucosidase) which play a key role in effective reduction of fat as well as carbohydrate absorption. Orlistat and acarbose have been a potent reversible inhibitor of pancreatic lipase³ and α-amylase and α-glucosidase⁴, respectively. However, undesirable side effects such as flatulence and diarrhea limit their use.⁵ Therefore, better alternative anti-obesity and anti-diabetic agents to prevent hyperlipidemia⁵ and hyperglycemia⁶ have been evaluated, especially from plants. A flavonoid called rutin, found abundantly in *Centella asiatica* (L.) Urb. (Umbelliferae)⁸,⁹, is reported to have several pharmacological properties including hypolipidemic,⁴⁻¹² hypoglycemic,¹³, and digestive enzyme inhibitory activities, *i.e.*, lipase, α-amylase and/or α-glucosidase¹⁴⁻¹⁷.

*C. asiatica*, an ethnomedicinal plant, contains variety of active compounds such as phenolics, triterpenoids, and flavonoids (catechin, rutin, naringin, quercetin, and luteolin).⁹ Anti-diabetic activity of *C. asiatica* ethanolic extract has been demonstrated in alloxan⁸ and streptozotocin¹⁹ induced diabetic rats. Inhibition of *C. asiatica* extract on carbohydrate digestive enzymes (α-amylase and/or α-glucosidase)²⁰⁻²² and lipase digestive enzymes (pancreatic lipase and pancreatic cholesterol esterase)²³ has been demonstrated *in vitro*. Moreover, *C. asiatica* extract exerted hypotriglyceridemic action and attenuated food intake and body weight in hydrogen peroxide (*H₂O₂*)-induced oxidative stress rats.⁹ At present, hypolipidemic and hypoglycemic effects of *C. asiatica* extract and its constituent, rutin, and its possible mechanisms in lipid emulsion-induced hyperlipidemic rats have been studied for the first time.

**Materials and Methods**

*Preparation of plant extract—* *C. asiatica* was collected from Nakhon Pathom province, Thailand, and authenticated by the Department of National Park, Wildlife and Plant Conservation, Thailand. The voucher specimen (BKF No.184894) was deposited in the Forest Herbarium, Department of National Park, Wildlife and Plant Conservation, Thailand. The edible parts of *C. asiatica* were washed, and dried at
45-50 °C for 4 h and blended. The dried powder was then extracted by maceration method with 80% ethanol for 7 days. The filtrate was concentrated using a rotary evaporator before lyophilisation. The crude extract obtained was stored at -20 °C until further use.

The extract or rutin (Sigma Aldrich, St. Louis, USA) was dissolved in DMSO to give concentrations ranging 62.5-4000 µg/mL in pancreatic lipase and α-amylase inhibitory assay and 0.625-640 µg/mL in α-glucosidase inhibitory assay.

**Chemicals and reagents**—Porcine pancreatic lipase (Sigma Aldrich-7545), porcine pancreatic α-amylase (Sigma Aldrich-3176), yeast α-glucosidase (Sigma Aldrich-5003), orlistat, acarbose, p-nitrophenyl butyrate (p-NPB), starch soluble, 4-nitrophenyl-α-D-glucopyranoside (PNPG), and rutin trihydrate were purchased from Sigma Aldrich Chemical Ltd. (St. Louis, USA). Lipid emulsion (20% Intralipid) was purchased from Sino-Swed Ltd. (China). All other chemicals were of reagent grade.

**Determination of total phenolic content**—The total phenolic content was determined according to Minussi et al.²⁴. Briefly, the *C. asiatica* extract was dissolved in 10% ethanol. The reaction mixtures consisted of *C. asiatica* solution (200 µL) and 4 mL of 2% Na₂CO₃ (BDH Ltd., UK) were mixed. Two minutes later, 200 µL of Folin-Ciocalteu reagent was added and incubated at room temperature for 30 min. The mixtures were measured for absorbance using a spectrophotometer (CECIL 1011, England) at 750 nm. The total phenolic compounds were expressed as gallic acid equivalents in mg/g dry extract.

**Determination of rutin in *C. asiatica* extract by HPLC**—The HPLC was performed to analyze the rutin content in *C. asiatica* extract. The HPLC apparatus consisted of a binary pump, an autosampler, a column oven, and UV-VIS detector system (Shimadzu LC-10AD, Kyoto, Japan) coupled with a Phenomenex C18 column (4.6 × 250 mm, and 5 µm particle size, Cheshire, UK). The mobile phase consisted of acetonitrile (solvent A) and 1% (v/v) acetic acid in water (solvent B). The linear gradient program was as follows: 0% A and 100% B in the first 0 min, held for 5 min, changed to 40% A and 60% B over 35 min, then changed to 80% A and 20% B, held for 10 min, changed to 100% A and 0% B, held for 10 min. The mobile phase flow rate was 1 mL/min, and the UV detector was monitored at 254 nm. The injection volume was 20 µL. All the chromatographic operations were carried out at ambient temperature. All quantitative analysis were made using the external standard method.

**Measurement of pancreatic lipase inhibitory activity**—The pancreatic lipase inhibitory activity was measured adopting Kim *et al.*²⁵ with little modification. Briefly, 20 µL of *C. asiatica* extract, rutin or orlistat (positive control) mixed with 6 µL of pancreatic lipase solution (2.5 mg/mL) in buffer containing 10 mM morpholinepropanesulfonic acid (MOPS) and 1 mM EDTA, pH 6.8. The mixture was added to 169 µL of Tris buffer (100 mM Tris-HCl and 5 mM CaCl₂, pH 7.0) and incubated at 37 °C for 30 min. After that, 5 µL of the substrate solution (10 mM p-NPB) in dimethyl formamide) was added and incubated at 37 °C for 30 min. Lipase activity was determined by measuring the hydrolysis of p-NPB to p-nitrophenol at 405 nm using microplate reader (Benchmark plus, Japan). Inhibition of pancreatic lipase activity was calculated according to the formula:

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

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.

**Measurement of α-amylase inhibitory activity**—Activity of α-amylase was determined by measuring the amount of maltose released using colorimetric method with a slight modification.²⁶ Briefly, 50 µL of *C. asiatica* extract, rutin or acarbose (positive control) mixed with 50 µL of α-amylase (0.5 units/mL). The mixture was incubated at 25 °C for 30 min. Then, 100 µL of starch solution (0.5%, w/v) was added and incubated at 25 °C for 3 min. After that, 100 µL of the colour reagent (20 mL of 96 mM 3, 5 dinitrosalicylic acid, 5.31 M sodium potassium tartrate in 2 M sodium hydroxide (8 mL) and 12 mL of double deionized distilled (DDD) water) was added and incubated at 85 °C for 15 min. The mixture was then cooled and 900 µL of DDD water was added. The final mixture was measured at 540 nm using microplate reader. The inhibition percentage of α-amylase activity was assessed by the formula:

\[
\text{Inhibition (\%) = } 100 \times \frac{(\Delta A_{\text{Control}} - \Delta A_{\text{Sample}})}{\Delta A_{\text{Control}}}
\]

\[
\Delta A_{\text{Control}} = A_{\text{Test1}} - A_{\text{Blank1}};
\]

\[
\Delta A_{\text{Sample}} = A_{\text{Test2}} - A_{\text{Blank2}}
\]

where \(A_{\text{Test1}}\) and \(A_{\text{Test2}}\) are defined as the absorbance of DMSO and sample with α-amylase enzyme. \(A_{\text{Blank1}}\) and \(A_{\text{Blank2}}\) are defined as the absorbance of DMSO water and sample without α-amylase enzyme.
**Measurement of α-glucosidase inhibitory activity**—Si et al.\(^27\) was followed for determination of α-glucosidase activity using \(p\)-PNPG as a substrate. The mixture consisted of 25 μL of 3 mM reduced glutathione, 250 μL of 0.067 M potassium phosphate buffer (pH 6.8), 25 μL of 0.3 unit/mL of yeast α-glucosidase, and 25 μL of *C. asiatica* extract, rutin or acarbose (positive control). The mixture was incubated at 37 °C for 10 min, and repeated after adding 25 μL of 10 mM PNPG. The reaction was stopped by adding 400 μL of 0.1 M sodium carbonate solution. \(p\)-Nitrophenol released from PNPG was detected at 400 nm using microplate reader. The inhibition percentage of α-glucosidase activity was assessed by the formula:

\[
\text{Inhibition} \, (\%) = \left(1 - \frac{A_{0}}{A_x}\right) \times 100
\]

where \(A_0\) is defined as the absorbance of DMSO and sample with α-glucosidase enzyme; \(A_x\) without α-glucosidase enzyme.

**IC\(_{50}\) values**—The concentration of *C. asiatica* extract, rutin, orlistat, and acarbose required to inhibit 50% of enzyme activity was defined as the IC\(_{50}\) value. The percent of pancreatic lipase, α-amylase, and α-glucosidase inhibitory activities were plotted against the sample concentration and a logarithmic regression curve established in order to determine the IC\(_{50}\) value.

**Animals and experiment protocol**—Eight weeks-old male Wistar rats (40) obtained from Institutional Animal Care, Suranaree University of Technology, were housed under standard laboratory conditions (12:12 h dark-light cycle and 20±1 °C ambient temperature) with free access to food and water. The experiment protocol was reviewed and approved by the institutional authority. Groups of 8 rats were orally administered with lipid emulsion (4 mL/kg) alone to serve as control (Group I); lipid emulsion + orlistat (45 mg/4 mL/kg, Group II); lipid emulsion + rutin (1000 mg/4 mL/kg, Group III); lipid emulsion + *C. asiatica* extract (1000 mg/4 mL/kg, Group IV), and lipid emulsion + *C. asiatica* extract (2000 mg/4 mL/kg, Group V). The lipid emulsion was composed of purified soybean oil (200 g), purified egg phospholipids (12 g), and glycerol anhydrous (22 g). Blood samples were collected from the tail vein into heparinized tube at 0, 1, 2, 3, and 4 h after the oral administration of each treatment and centrifuged at 5000 \(\times\) g for 5 min. Plasma levels of triglyceride (TG), total cholesterol (TC), glucose, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined using an automated blood analyzer (Hitachi 911, Japan). Animals of all groups were observed continuously for any behavioural changes, general toxicity signs, and mortality during the experimental period. At the end of the experiment (4 h after each treatment), all of the animals were sacrificed with an overdose of sodium pentobarbital.

**Statistical analysis**—The results are expressed as mean±SE. All statistical analysis was performed using the SigmaStat 3.5 software (Systat Software Inc., San Diego, USA). The difference of IC\(_{50}\) values were analyzed by one-way analysis of variance (ANOVA) followed by the Tukey’s test. The differences of plasma TG, TC, glucose, AST, and ALT levels were analyzed by two-way repeated measures ANOVA followed by Tukey’s test. Probability of value less than 0.05 \((P<0.05)\) was considered to denote significant difference between groups.

**Results**

**Total phenolic content including rutin in *C. asiatica* extract**—Total phenolic content in *C. asiatica* extract was 97.75 mg/g gallic acid. The analysis of rutin content in the extract by HPLC was obtained from a standard curve \((y = 38671x+426.1)\) with a correlation coefficient of 0.9990. *C. asiatica* extract contained 3.9% of rutin.

**Inhibitory effects of *C. asiatica* extract and rutin on pancreatic lipase, α-amylase and α-glucosidase**—The inhibitory effect of *C. asiatica* extract, rutin, orlistat, and acarbose on pancreatic lipase, α-amylase, and α-glucosidase are presented in Table 1. The IC\(_{50}\) values of *C. asiatica* extract, rutin, and orlistat for pancreatic lipase inhibition were 759.14±3.63, 1412.24±5.38, and 0.61±0.16 μg/mL, respectively.

**Table 1**—Inhibitory effects of *C. asiatica* extract, rutin, orlistat, and acarbose on pancreatic lipase, α-amylase, and α-glucosidase activities.

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>C. asiatica extract</th>
<th>Rutin</th>
<th>Orlistat</th>
<th>Acarbose</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC(_{50}) (μg/mL)</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>pancreatic lipase</td>
<td>759.14±3.63 (^a)</td>
<td>1412.24±5.38 (^b)</td>
<td>0.61±0.16</td>
<td>NT</td>
</tr>
<tr>
<td>α-amylase</td>
<td>536.51±8.80 (^b)</td>
<td>513.09±5.11</td>
<td>NT</td>
<td>113.25±2.72</td>
</tr>
<tr>
<td>α-glucosidase</td>
<td>42.27±1.66</td>
<td>47.10±7.64</td>
<td>NT</td>
<td>34.07±3.69</td>
</tr>
</tbody>
</table>

\(^a\)Compared to control (orlistat or acarbose); \(^b\)rutin. IC\(_{50}\) values are the concentration required to inhibit enzyme activities by 50%. NT indicates no test.
Pancreatic lipase inhibitory activities of the extract and rutin were significantly less than that of orlistat. The *C. asiatica* extract showed significantly higher pancreatic lipase inhibitory activity than rutin. The IC\(_{50}\) values of *C. asiatica* extract, rutin, and acarbose for α-amylase activities were 536.51±8.80, 513.09±5.11, and 113.25±2.72 µg/mL, respectively. The *C. asiatica* extract and rutin showed comparatively significant inhibition against α-amylase compared to acarbose. The IC\(_{50}\) values of *C. asiatica* extract, rutin, and acarbose for α-glucosidase activities were 42.27±1.66, 47.10±7.64, and 34.07±3.69 µg/mL, respectively. Surprisingly, α-glucosidase inhibitory activities of the *C. asiatica* extract, rutin, and acarbose were equipotent.

**Effects of *C. asiatica* extract, rutin, and orlistat on plasma TG, TC and glucose**—Changes in plasma TG, TC and glucose levels following an oral administration of lipid emulsion alone, lipid emulsion plus either *C. asiatica* extract, rutin, or orlistat are shown in Table 2. Significant increases in plasma TG levels were found after 1, 2, and 3 h of oral administration of the lipid emulsion alone compared to 0 h. After 3 h, the plasma TG levels induced by lipid emulsion was significantly reduced by *C. asiatica* extract (1000 and 2000 mg/4 mL/kg), rutin and orlistat. In comparison, rats treated with lipid emulsion + orlistat or rutin showed no significant difference in the plasma TG levels after 3 h as well as the rats treated with *C. asiatica* extract, respectively. Increase in plasma TC levels with peak at 3 h was found in lipid emulsion alone compared to 0 h (Group I) which was significantly suppressed by *C. asiatica* extract (1000 and 2000 mg/4 mL/kg), rutin and orlistat compared to lipid emulsion alone. However, there was no significant difference in the plasma TC levels of the rats treated with lipid emulsion + orlistat or

Table 2—Plasma levels of TG, TC, Glucose, AST and ALT (mg/dL) after an oral administration of lipid emulsion alone, lipid emulsion plus either *C. asiatica* extract, rutin, or orlistat, respectively

<table>
<thead>
<tr>
<th>Groups</th>
<th>0 h</th>
<th>1 h</th>
<th>2 h</th>
<th>3 h</th>
<th>4 h</th>
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<tr>
<td></td>
<td>65.06±5.50</td>
<td>88.00±15.12(^{a})</td>
<td>97.17±13.71(^{b})</td>
<td>118.80±8.83(^{b})</td>
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<td></td>
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<td>90.60±1.82(^{b})</td>
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<td>141.99±6.63</td>
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<td>78.52±4.81(^{c})</td>
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<td>52.14±5.75</td>
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</tbody>
</table>

\(^{a}\)P<0.05 and \(^{b}\)P<0.001 compared to 0 h within the group, respectively.

\(^{c}\)P<0.05 and \(^{d}\)P<0.001 compared to lipid emulsion alone group within time, respectively.

Group I: lipid emulsion (4 mL/kg); Group II: lipid emulsion + orlistat (45 mg/4 mL/kg); Group III: lipid emulsion + rutin (1000 mg/4 mL/kg); Group IV: lipid emulsion + *C. asiatica* extract (1000 mg/4 mL/kg); Group V: lipid emulsion + *C. asiatica* extract (2000 mg/4 mL/kg).
rutin as well as the groups treated with *C. asiatica* extract after 3 h (Table 2). An oral administration of lipid emulsion did not alter plasma levels of glucose (Table 2). However, after 3 h of treatment, the plasma glucose levels showed significant decrease in the groups treated with *C. asiatica* extract as well as rutin. However, there was no significant difference in plasma glucose levels of the rats treated with lipid emulsion plus *C. asiatica* extract (1000 mg/4 mL/kg) compared to the rats treated with lipid emulsion + rutin. The group II (lipid emulsion + orlistat) also showed significant decrease in plasma glucose level 4 h after treatment.

**Effects of C. asiatica extract, rutin, and orlistat on plasma AST and ALT**—During the experimental period of 4 h, plasma levels of hepatic enzymes AST and ALT did not show any significant change after a single oral dose of lipid emulsion alone, lipid emulsion plus either *C. asiatica* extract, rutin, or orlistat (Table 2). In addition, no death or adverse toxic signs (changes in behavioral patterns, breathing, salivation, lethargy, diarrhea, and alertness) occurred in any of the groups.

**Discussion**

The present study has demonstrated the significance of *C. asiatica* extract as a potent hypolipidemic and hypoglycemic agent in lipid emulsion-induced hyperlipidemic rats. These effects may be attributed to the flavonoids found in *C. asiatica*, in particular rutin a known inhibitory agent of pancreatic lipase, α-amylase, and α-glucosidase. These findings indicated that the increases in plasma levels of TG induced by lipid emulsion were suppressed by *C. asiatica* extract including its constituent rutin probably due to direct inhibition of pancreatic lipase activity. The present findings are in accordance with the previous studies, revealing a potent hypolipidemic activity of *C. asiatica* extract *in vitro* and *in vivo* in rats fed high fat-fed diets and high fat-fed rats. The present study also demonstrated the reduction of plasma TC level caused by *C. asiatica* extract and rutin that may involve in the pancreatic cholesterol esterase inhibitory activity as well as the inhibition of cholesterol micellization and binding of bile acids, decreased 3-hydroxy-3-methylglutaryl-CoA reductase activity, and/or decreased absorption of dietary cholesterol. The results are significant in the sense that the *C. asiatica* extract (1000 mg/4 mL/kg) exhibited a greater inhibitory effect on plasma TG and TC levels than rutin at 3 h (Table 2) similar to rats fed a high cholesterol supplemented with rutin (1000 mg/kg) and also in rats orally administered with rutin at 1000 mg/kg for 22 days.

Orlistat was used as a positive control for inhibitory effects against gastric and pancreatic lipases, resulting in suppressing the digestion and absorption of fat and promoting excretion of ingested fat leading to weight loss. Orlistat at the dose of 45 mg/kg was reported to cause significant reduction of the increased plasma TG levels following lipid emulsion, which was similar to the present study. Treatments with *C. asiatica* extract, rutin and orlistat showed significantly reduced plasma TG and TC levels at 3 h. The effects of *C. asiatica* extract were higher than that of rutin and orlistat demonstrating that the hypotriglyceridemic and hypocholesterolemic activities of *C. asiatica* extract are better than rutin and orlistat. *C. asiatica* extract and rutin significantly suppressed plasma glucose levels in lipid emulsion fed rats, similar to acarbose, a positive control. Earlier studies have shown the potent hypoglycemic effect of *C. asiatica* extract both *in vitro* and *in vivo*. Its active compound rutin also possesses a potent hypoglycemic effect *in vitro* and *in vivo*. Thus, the mechanism underlying hypoglycemic effects of *C. asiatica* extract and rutin is possibly the reducing postprandial hyperglycemia by slowing down the digestion of carbohydrates and the absorption of glucose. Moreover, this study demonstrated that orlistat could reduce the plasma glucose level which may involve in reduction of plasma non-esterified fatty acid concentration, improvement of insulin sensitivity and stimulation of glucagon-like peptide-1 (GLP-1) secretion from small intestine.

In the present oral acute toxicity observation up to 4 h, *C. asiatica* extract at doses of 1000 and 2000 mg/4 mL/kg and rutin at a dose of 1000 mg/4 mL/kg have been found to be safe without any toxicity signs or mortality. This is in agreement with Roopesh et al. who demonstrated nil mortality in rats administered with ethanolic extract of *C. asiatica* up to 5000 mg/kg. In rats, rutin at a dose of 1000 mg/kg revealed no toxicity signs following 22-day oral administration. Additionally, no significant alteration in plasma levels of hepatic injury biomarker enzymes (AST and ALT) following *C. asiatica* extract and rutin treatments suggest that the *C. asiatica* extract and rutin are non-toxic and generally safe.

**Conclusion**

In summary, *C. asiatica* extract and its active compound rutin may provide a safe, natural, and cost-
effective treatment for hyperlipidemia and hyperglycemia. Further investigation is needed to study anti-obesity mechanisms of Centella asiatica extract in animal model.

Conflict of Interest
The authors have declared that there is no conflict of interest.

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