Strong Antihyperglycemic Effects of Water-Soluble Fraction of Brazilian Propolis and Its Bioactive Constituent, 3,4,5-Tri-O-cafeoylquinic Acid

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To clarify the suppression of postprandial blood glucose rise via α-glucosidase (AGH) inhibitory action by natural compounds, propolis was examined in this study. A single oral administration of propolis extract (50% methanol fraction on XAD-2 column chromatography) in Sprague–Dawley rats demonstrated a potent antihyperglycemic effect with the significant AUC reduction of 38% at a dose of 20 mg/kg compared to that of controls. Among the active compounds isolated from the fraction, 3,4,5-tri-c-caffeoylquinic acid was found to be a prominent candidate that exerts the effect and shows a strong maltase-specific inhibition with an IC50 value of 24 μM. In addition, the noncompetitive inhibition power apparently increased with the number of caffeoyl groups bound to quinic acid.

Key words α-glucosidase; c-caffeoylquinic acid; propolis; antihyperglycemic effect; diabetes

Noninsulin-dependent diabetes mellitus (NIDDM) is one of the serious lifestyle-related disorders, and clinical trials have been performed to moderate excess postprandial blood glucose level (BGL), to improve insulin resistance, or to enhance insulin secretion. Prevention of the development of its subsequent combined diseases such as retinopathy, neuropathy, and cataracts is also of great importance clinically. In the field of food science, much interest has been focused on the development of alternative medicinal foods which includes screening of natural bioactive compounds with the ability to delay glucose absorption. This is because any control of postprandial BGL rise by antihyperglycemic foods would be of benefit improving the quality of life (QOL) of people with borderline NIDDM. Such physiologic functions as α-glucosidase, α-amylase, or glucose transport inhibition have been candidates to flatten the postprandial BGL rise by natural compounds. Among these, α-glucosidase (AGH, EC 3.2.1.20) inhibition may fulfill the need, since it has been confirmed that daily intake of therapeutic AGH inhibitors such as acarbose or voglibose is effective in moderating hyperglycemia in borderline NIDDM.

In a series of AGH inhibition studies using food components, we have clarified some natural active compounds that are closely associated with the suppression of glucose production from dietary carbohydrates through the retardation of maltase activity. Diaxylated anthocyanins such as peorlagordinin 3-O-(2-O-(6-O-(E-3-O-(β-D-glucopyranosyl)-caffeyl)-β-D-glucopyranosyl)-6-O-E-cafeyl-β-D-glucopyranoside)-5-O-β-D-glucopyranoside (IC50: 60 μM), piscinon 3-O-(2-O-(6-O-E-(E-3-O-(β-D-glucopyranosyl)-6-O-E-cafeyl-β-D-glucopyranoside)-5-O-β-D-glucopyranoside) (IC50: 200 μM), and 6-O-cafeoylsphorosine, which occurs in an acetylated moiety of the anthocyanins (IC50: 699 μM) are effective natural maltase inhibitors that we have identified. However, there is no report on natural inhibitors with strong maltase inhibitory activity comparable to therapeutic drugs (IC50: acarbose, 0.426 μM; voglibose, 0.0055 μM).

In this study, we used Brazilian propolis as a new source of AGH inhibitors. Propolis is a resinous substance collected by honeybees (Apis mellifera) from exudates and buds of plants and mixed with secreted beeswax. People have used propolis as a folk medicine from ancient times. Even though propolis has diverse physiologic functions such as antioxidant, antibacterial, and antiinflammatory effects, there are no reports on its antidiabetic effect due to its great variability of constituents including flavonoids, phenolic acids, cinnamic acid derivatives, terpenes, lignans, etc. Hence the objective of the present study was to clarify any antihyperglycemic effects of propolis constituents in rats to identify strong AGH inhibitors in propolis and to confirm their potential antidiabetic effects.

MATERIALS AND METHODS

Materials. Brazilian propolis was a gift from Yamada Apiculture Center Inc. (Okayama, Japan), which is an ethanol-extracted solution from propolis and contains 55% propolis extract as a solid content. α-Glucosidase (AGH, EC 3.2.1.20) from rat intestinal acetone powder and human saliva α-amylase (EC 3.2.1.1) were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A). CNBr-activated Sepharose 4B was purchased from Pharmacia Biotech AB (Uppsala, Sweden). Caffeic acid, chlorogenic acid, and quinic acid were purchased from Nacalai Tesque Co. Ltd. (Kyoto, Japan). Other reagents were of analytical grade and used without further purification.

Instruments. 1H- and 13C-NMR spectra were recorded on a JMN A400 NMR instrument (JEOL) at 30°C. The solvent used was deuterium methanol-d4 (MeOD). Tetramethylsilane was used as an internal standard. Electron spray-izationation (ESI)-MS was performed on a Waters 2790 micromass ZQMS system (capillary voltage, +3 kV; cone voltage, +15.0 V) (Tokyo, Japan).

Preparation of Propolis Extract. One gram of Brazilian propolis ethanol-extracted solution with a content of 55% as

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a solid propolis extract was first extracted with 6 ml of n-hexane: water (1:1). After stirring vigorously for 10 min, the mixture was centrifuged at 1500 \(g\) for 10 min at room temperature, separated into water and hexane layers (denoted as WP and HP, respectively). Both layers were collected to dryness (yield: WP, 90.8 mg/g of propolis; HP, 146.3 mg/g of propolis). In this study, the unit of mg/g of propolis represents a content per gram of solid propolis extract. The WP extract was then dissolved in 5 ml of water. An aliquot (1 ml) of the solution was then subjected to Amberlite XAD-2 column chromatography (Supelco Inc., Bellefonte, PA, U.S.A., 0.25×32 cm), and successively eluted with 250 ml of water, 50% methanol, and methanol (yield of each fraction was 28.9, 18.9, 4.1 mg/g of propolis, respectively). Each fraction denoted as WP-A0, WP-A50, and WP-A100 for water, 50% methanol, and methanol extraction, respectively, was collected to dryness and stored at -4°C until use.

**Isolation of Active Compounds** A fraction with AGH inhibitory activity was applied to an HPLC system (LC-2000plus intelligent pump system, JASCO Co., Japan) on an ODS column (Cosmosil 5C18-ARII, \(\phi 4.6\times250 \text{mm}, \text{Nacalai Tesque}\)) with the linear gradient solvent system of CH_3CN (10—70%, 60 min) in 0.1% trifluoroacetic acid at a flow rate of 0.3 ml/min at 400 nm. Then, 100 \(\mu l\) of the active WP-A50 fraction (20 mg/ml) was applied to reverse-phase HPLC, and the observed peaks were individually collected to dryness. The obtained dried samples were then resolved in 100 \(\mu l\) of a model intestinal fluid solution, followed by the immobilized AGH assay as a substrate of maltose (10 mM) to evaluate the maltase inhibition of each peak. As a result of the AGH assay, we obtained five peaks with maltase inhibition from the WP-A50 fraction; peak 1: brown powder, 50.0 min on the HPLC retention, 0.61 mg/g of propolis in yield; peak 2: light yellow powder, 51.1 min, 1.25 mg/g of propolis; peak 3: yellow powder, 52.3 min, 2.20 mg/g of propolis; peak 4: yellow powder, 55.5 min, 0.29 mg/g of propolis; peak 5: yellow powder, 59.0 min, 0.29 mg/g of propolis. As a result of MS and NMR analyses, we identified three constituents from yellow powder, 59.0 min, 0.29 mg/g of propolis. As a result of MS and NMR analyses, we identified three constituents from the extracted sample. The WP and HP fractions. As shown in Table 1, the propolis extract contained a content per gram of solid propolis extract. The WP and HP fractions. As shown in Table 1, the propolis extract contained a content per gram of solid propolis extract.
preferentially inhibited maltase action rather than sucrase and \( \alpha \)-amylase by a factor of \( ca. 5 \). This indicated that propolis may have the potential ability to delay glucose release from maltose or disaccharides at the small intestinal membrane. As many investigators have already demonstrated, propolis is a beneficial medicinal resource with antibacterial, antiviral, and antiinflammatory effects as well as immunomodulating and antihepatotoxic effects.\(^{13-15} \) In addition to these antiviral, and antifibrinogen actions, propolis is a beneficial medicinal resource with antibacterial, membrane. As many investigators have already demonstrated, propolis may have the potential ability to delay glucose release from dietary carbohydrates. The maltase inhibitory activity with an \( IC_{50} \) value of 1.0 mg/ml was, however, still weaker (\( ca. 1/5 \)-fold lower) than those of other active extracts with in vivo antihyperglycemic effect such as the extract from storage roots of purple sweet potato (\( IC_{50} \), 0.26 mg/ml),\(^{10,19} \) the extract from Scarlett O’Hara flower (\( IC_{50} \), 0.17 mg/ml), and the extract from green tea (\( IC_{50} \), 0.22 mg/ml).\(^{10} \) Thus, to enhance the maltase-inhibitory activity of propolis extract, liquid extraction was then performed. As a result of water–hexane extraction of propolis extract, successful enhancement of maltase inhibitory activity was achieved for the WP fraction, whereas no or less enhancement of sucrase and \( \alpha \)-amylase inhibition was observed (Table 1). A three-fold higher increase in maltase inhibition of the WP fraction than that of the propolis extract was achieved with the treatment, which is comparable to those of the above-mentioned active extracts. No inhibition of the HP fraction against the hydrolylases and the enhanced activity of WP fraction clearly indicated that more than one active propolis constituent, likely to be polar or hydrophilic in nature, was closely associated with AGH (maltase) inhibition.

Thus our next strategy to enhance the biological activity of the WP fraction and identify the maltase-inhibitory constituents in it was performed with XAD-2 column chromatography with methanol. A stepwise elution of WP with water, 50% methanol, and methanol resulted in sufficient separation into three active fractions, as shown in Table 2. At a concentration of 1.0 mg/ml of each fraction, all fractions showed a high maltase inhibitory ratio of >40% with the descending order of 50% methanol fraction > methanol fraction > water fraction. In particular, the 50% methanol fraction (WP-A50) was found to have the most potent maltase-inhibitory activity, indicating that prominent bioactive constituents in propolis extract were collected in the WP-A50 fraction. The results of \( iAGH \)-inhibitory activity (\( IC_{50} \)) of WP-A50 shown in Table 2 also revealed a powerful and preferential maltase-inhibitory activity of 0.049 mg/ml.

This strong maltase inhibition was comparable to that of the methanol extract of \( Cassia auriculata \) flower (\( IC_{50} \), 0.023 mg/ml), with which the postprandial BGL rise in rats was significantly decreased only at a dose of 5 mg/kg.\(^{20} \) Hence, in the next step, a rat experiment with the WP-A50 fraction was performed to confirm and clarify its antihyperglycemic effects.

### Antihyperglycemic Effects of WP-A50 Fraction

Figure 1A represents the incremental BGL curve following maltose ingestion (2 g/kg) when the WP-A50 fraction was orally administered to SD rats at a dose of 20 mg/kg. The BGL curve in the WP-A50 group was lower than that in the control group; significant BGL reductions of 45.1 mg/dl and 28.8 mg/dl were observed at 30 and 60 min, respectively. The area under the curve (\( AUC_{0-120}\text{min} \)) of WP-A50 ingestion (80.7±4.2 mg·h/dl) was also markedly lower than that of the controls (130.7±8.3 mg·h/dl). These findings strongly suggest that the WP-A50 fraction may control or delay the postprandial BGL rise and effective in improving deficient insulin secretion.\(^{1} \) Although there are no reports on the in vivo antihyperglycemic effects of propolis, it was reported by Gray and Flatt\(^{21} \) that eucalyptus, which is thought to be one of the origins of propolis, had an antihyperglicemic action as well as antiobesity effect in streptozotocin-induced diabetic mice. They concluded that the effects of eucalyptus were due to the enhancement of insulin secretion and muscle glucose uptake as a result of long-term treatment of the mice. However, they did not refer to the physiologic function of suppressing glucose production in the gut. In contrast, as shown in Fig. 2, the WP-A50 fraction significantly (\( p<0.05 \) vs. control) reduced the rise in serum insulin level at 30 min in response to maltose ingestion (insulin \( \text{control} -30\text{min} \), 15.7±1.6 \( \mu U/ml \) of serum; insulin \( \text{WP-A50} -30\text{min} \), 9.1±1.8 \( \mu U/ml \) of serum). This result suggests that the WP-A50 fraction suppressed the BGL rise in SD rats via lowering of glucose absorption, but not promotion of insulin secretion.

Figure 3 shows the result of a dose-dependent experiment with WP-A50 in SD rats when maltose was ingested. Acorbose at a dose of 3 mg/kg was used as a positive control. As shown in Fig. 3, the WP-A50 fraction dose dependently suppressed the postprandial BGL rise during the BGL incremental period of 30 and 60 min after administration. This indicates that the antihyperglycemic effects of the WP-A50 fraction from propolis continued within this acute experimental period of 120 min. The \( AUC_{0-120}\text{min} \) obtained at each dosage revealed that 5 mg/kg of the WP-A50 fraction was sufficient to reduce the postprandial BGL rise by 75.5%.

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**Table 1. \( \alpha \)-Glucosidase\(^{a} \) and \( \alpha \)-Amylase Inhibitory Activities of Propolis Extracts**

<table>
<thead>
<tr>
<th>Elution</th>
<th>Yield(^{b} ) (%)</th>
<th>Inhibitory ratio(^{c} ) (%)</th>
<th>( IC_{50} ) value (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>31.8</td>
<td>41.9</td>
<td>0.049</td>
</tr>
<tr>
<td>50% Methanol</td>
<td>20.9</td>
<td>76.2</td>
<td>0.049</td>
</tr>
<tr>
<td>Methanol</td>
<td>4.5</td>
<td>56.3</td>
<td>0.049</td>
</tr>
</tbody>
</table>

\(^{a} \) \( \alpha \)-Glucosidase inhibitory assay was performed on the immobilized assay system by using 10 m M maltose or 45 m M sucrose. \(^{b} \) Solid propolis extract, c) WP, water phase. \(^{c} \) HP, hexane phase. Each fraction was obtained by water–hexane (1 : 1) extraction of the propolis extract. \(^{d} \) N.I.; no inhibition.

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**Table 2. \( \alpha \)-Glucosidase Inhibitory Profile of Water-Soluble Fraction of Propolis\(^{a} \)**

<table>
<thead>
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\(^{a} \) Water-soluble fraction of propolis extract was separated into water, 50% methanol and methanol fractions on an Amberlite XAD-2 column chromatography, and each was subjected to an immobilized AGH assay. b) Yield of each eluate against applied water-soluble fraction of propolis. \(^{c} \) Inhibitory ratio (%) of each eluate against maltase activity was determined at a concentration of 1.0 mg/ml. \(^{d} \) — not measured.

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This shows the result of a dose-dependent experiment with WP-A50 in SD rats when maltose was ingested. Acorbose at a dose of 3 mg/kg was used as a positive control. As shown in Fig. 3, the WP-A50 fraction dose dependently suppressed the postprandial BGL rise during the BGL incremental period of 30 and 60 min after administration. This indicates that the antihyperglycemic effects of the WP-A50 fraction from propolis continued within this acute experimental period of 120 min. The \( AUC_{0-120}\text{min} \) obtained at each dosage revealed that 5 mg/kg of the WP-A50 fraction was sufficient to reduce the postprandial BGL rise by 75.5%.
compared with the control group ($p < 0.05$ vs. control): $AUC_{0-120 \text{ min}}$ of control, 130.7 ± 8.3 mg·h/dl; 5 mg/kg, 98.7 ± 3.0; 10 mg/kg, 91.7 ± 2.2; 20 mg/kg, 80.7 ± 4.2; acarbose (3 mg/kg), 76.1 ± 13.0. Based on the $AUC$ data, the WP-A50 dose required to achieve 50% suppression of the BGL rise ($ED_{50}$) was tentatively estimated to be about 33 mg/kg. Seri et al.\textsuperscript{22} reported that D-arabinose, a specific sucrase inhibitor, potently reduced BGL, with an $ED_{50}$ value of 18.5 mg/kg, as did D-xylose,\textsuperscript{23} although the experimental conditions were different from those in the present study. Water extracts of Sophora plants were also found to have strong antihyperglycemic effects with an $ED_{50}$ value of 6.4 mg/kg in rats that ingested sucrose.\textsuperscript{24} However, most antihyperglycemic studies of natural compounds focused on sucrase inhibition. Although the predicted antihyperglycemic activity of WP-A50 in maltose-fed SD rats was still lower than that of acarbose ($ED_{50}$, 3.1 mg/kg), no other natural maltase-inhibitory extracts or constituent(s) comparable to the propolis-induced BGL reduction activity were reported so far, except for the methanol extract of Ranawara ($ED_{50}$, 4.9 mg/kg).\textsuperscript{20}

Figures 1B and C shows the glycemic responses in SD rats when sucrose or glucose was ingested, respectively. As a result, neither sucrose nor glucose ingestion with the WP-A50 fraction in rats affected the postprandial BGL curve. No significant difference between the curves with and without the fraction in sucrose-fed rats was in fair agreement with the results in Table 2, suggesting that WP-A50 no longer has a BGL-lowering effect with sucrase inhibition. An additional interesting result that WP-A50 following glucose ingestion did not alter the postprandial BGL strongly suggests that the antihyperglycemic effect is achieved by restrictive maltase inhibition, not by inhibiting sucrase activity and glucose transport in the small intestinal membrane via the Na\textsuperscript{+}/glucose cotransporter.

Identification of Active Constituents in WP-A50 Fraction Based on our in vivo results, the WP-A50 fraction...
derived from propolis appears to be one of the most effective natural antihyperglycemic dietary adjuncts. Therefore our next interest was to clarify the constituent(s) involved in specific maltase inhibition. An attempt to separate the active constituent(s) in the WP-A50 fraction was made using reverse-phase HPLC on an ODS column with a linear gradient solvent system of CH₃CN (10—70%, 60 min) in 0.1% TFA. As a result of the HPLC separation in combination with the iAGH inhibitory assay using maltose as a substrate for all detected peaks, we found five peaks with inhibitory activity in the fraction with the HPLC retention time of 50.0 min for peaks 1, 51.1 min for peak 2, 52.3 min for peak 3, 55.5 min for peak 4, and 59.0 min for peak 5. Using NMR and MS analyses, we successfully identified three active constituents from peaks 2, 3, and 5 as 3,5-di-CQA,16) 3,4-di-CQA,16) and 3,4,5-tri-CQA,17) respectively (Fig. 4). Unfortunately, peaks 1 and 4 remained unclear due to their low concentration and structural complexity. Peaks 1 and 5 were considered to be prominent candidates for inhibiting maltase activity based on their low concentration and potent inhibitory activity (<60%).

Useful information on propolis constituents was reviewed by Bankova et al.,15) who summarized 78 compounds such as flavonoids, lignands, cinnamic acid derivatives, terpenes, and sugars. The review also referred to the presence of caffeoylquinic acids in propolis, in which 3-CQA (chlorogenic acid), 4-CQA, 5-CQA, 4,5-di-CQA, 4,5-di-CQA methyl ester, and 3,4-di-CQA methyl ester as well as 3,4-di-CQA and 3,5-di-CQA that were identified in the present study were clarified as typical bioactive propolis constituents. However, to our knowledge, this was the first finding that 3,4,5-tri-CQA occurs in propolis, although the compound has been found in asteraceous plants such as sweet potato leaf.25,26)

**Antihyperglycemic Effects of Caffeoylquinic Acids**

The *in vitro* iAGH inhibitory activities (IC₅₀) of three CQAs isolated from propolis in addition to that of the therapeutic drugs acarbose and voglibose are summarized in Table 3. Those of caffeic acid, chlorogenic acid, and quinic acid are also presented to understand the mechanism underlying the AGH-inhibitory action of CQAs. Potent and preferential maltase inhibition was observed only for 3,4,5-tri-CQA (IC₅₀, 24 μM) among the CQA family. Although it has been reported that chlorogenic acid (3-CQA) acts as an antagonist of the intestinal glucose transporter,27) the acid had little influence on intestinal AGH in this study. Caffeic acid or quinic acid alone did not show any potent iAGH inhibitory activity; 3-CQA and two di-CQAs showed significant activity, but they were less active than tri-CQA. These findings clearly indicate that the caffeoyl group plays an important role in exerting iAGH-inhibitory activity and that an increasing number of caffeoyl groups enhances the maltase-inhibitory activity. Although tri-CQA was a potent sucrase inhibitor (IC₅₀, 0.57 mM) compared with other natural inhibitors such as d-xylose (IC₅₀, 1.19 mM),28) the much more potent maltase-inhibitory activity led us to the conclusion that tri-CQA is a maltase-specific inhibitor. On the basis of the total AGH-inhibitory activity (3,5-di-CQA, 1.3 units; 3,4-di-CQA, 2.2 units, 3,4,5-tri-CQA, 17.9 units; WP-A50 fraction, 385.7 units), we evaluated the magnitude of the AGH-inhibitory contribution of each isolated CQA to the overall inhibition of WP-A50 fraction to be 3,5-di-CQA (0.33%), 3,4-di-CQA (0.57%), and 3,4,5-tri-CQA (4.64%). This suggests that 3,4,5-tri-CQA is a prominent AGH-inhibitory contributor, but most active constituents involved in maltase inhibition remain in the WP-A50 fraction. Thus a further isolation study is needed to clarify the AGH-inhibitory constituents.

The inhibitory activity of tri-CQA was almost 1/6-fold lower than that of acarbose. Yoshikawa et al.29) reported that salacinol, which has *in vivo* antihyperglycemic effects and is one of the most powerful natural AGH inhibitors, exerted only ca. 1/3-fold lower maltase-inhibitory activity than that of acarbose, suggesting that the AGH inhibitory activity of tri-CQA is comparable to that of salacinol. Although they did not refer to the structural factor required for the strong competitive inhibition by salacinol, the attachment of the sulfate group might be a candidate, since sulfoquinnovosyl-diaclyglycerol with a sulfate group also showed a strong competitive AGH inhibition (Ki, 2.9 μM).29) In contrast, tri-CQA has no ionizing group so that the inhibitory mechanism underlying the action of tri-CQA would be different from that of the sulfates.

The Lineweaver–Burk plots for the hydrolysis of maltose by iAGH (maltase) in the presence or absence of tri-CQA 30 μM (Fig. 5) demonstrated that tri-CQA inhibits maltase action noncompetitively (Ki, 21 μM) as well as the two di-CQAs (Ki for both, 340 μM). Noncompetitive inhibitory action was also observed in other natural AGH inhibitors such as fructose,30) thio-fructofuranoside,31) caffeoylsophorose11) and diacylated anthocyanin.11) As Hauri et al.32) reported, sucrose and isomaltase form a dimeric complex at the intestinal membrane, into which only the C-terminal of the isomaltase subunit is anchored, and the sucrase subunit occurs as a complex form with the isomaltase subunit. Thus the noncompetitive and preferable maltase inhibition of tri-CQA would result from its binding to the sucrose subunit region in the sucrase-isomaltase complex. Additionally, it was concluded that the caffeoyl moiety and its number of caffeoyl groups enhances the maltase-inhibitory activity led us to the conclusion that 3,4,5-tri-CQA is a maltase-specific inhibitor. On the basis of the total AGH-inhibitory activity (3,5-di-CQA, 1.3 units; 3,4-di-CQA, 2.2 units, 3,4,5-tri-CQA, 17.9 units; WP-A50 fraction, 385.7 units), we evaluated the magnitude of the AGH-inhibitory contribution of each isolated CQA to the overall inhibition of WP-A50 fraction to be 3,5-di-CQA (0.33%), 3,4-di-CQA (0.57%), and 3,4,5-tri-CQA (4.64%). This suggests that 3,4,5-tri-CQA is a prominent AGH-inhibitory contributor, but most active constituents involved in maltase inhibition remain in the WP-A50 fraction. Thus a further isolation study is needed to clarify the AGH-inhibitory constituents.

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given based on the 1/6-fold lower maltase-specific inhibitory activity of tri-CQA than that of acarbose (Table 3) and its ED50 value of 3.1 mg/kg. As a result, tri-CQA exhibited antihyperglycemic effects in a dose-dependent manner with an AUC0—120 min of 100.4 ± 4.5 and 88.4 ± 14.4 mg · h/dl for the 10 and 20 mg/kg doses, respectively. At least 10 mg/kg of tri-CQA, at which a significant (p < 0.05) AUC reduction of 23.2% was observed comparable to that of controls (130.7 ± 8.3 mg · h/dl), was effective in eliciting the antihyperglycemic effects.

In the present study, we demonstrated that tri-CQA has a strong antihyperglycemic effect through the inhibition of intestinal maltase activity; the preferential maltase rather than sucrase inhibition would be of benefit in modulating postprandial BGL rise upon dietary carbohydrate intake. Early studies to evaluate the possible physiologic mechanism of the CQA family were performed mainly with mono- and di-CQAs. Immunomodulatory33) and hepatoprotective actions36,34) of mono- and/or di-CQAs were recognized as their bioactive functions through antioxidative activity25); di-CQAs, especially 3,4-di-CQA methyl ester, were reported to potently protect the liver injury in CCl4-induced rats.16) However, there were few reports on the physiologic functions of tri-CQA. Yoshimoto et al.26) reported that the tri-CQA isolated from sweet potato leaf exerted strong antimutagenicity against the reverse mutation in Trp-P-1-induced Salmonella typhimurium among the CQA family, and relationship between the effect and the number of caffeoyl groups was also observed. Those effects of 3,4,5-tri-CQA led us to believe that it would play an important role in maintaining homeostasis with potent physiologic functions. Further studies are needed to address the potential functions of tri-CQA.

In conclusion, the antihyperglycemic effects of propolis extract are attributed to the inhibition of intestinal AGH activity. The CQA family, in particular 3,4,5-tri-CQA is a possible candidate for exerting the effect via the specific retardation of maltase activity.

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