Original Article

Dietary Indigenous Cinnamon (*Cinnamomum osmophloeum*) Leaf Powder Reduces Plasma Lipid in Hypercholesterolemia Hamsters

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Abstract

Indigenous cinnamon (*Cinnamomum osmophloeum* Kaneh) is a native tree species in Taiwan and has been reported to have various bioactivities including insecticidal, larvicidal, and antimicrobial effects. The chemical finger print of *C. osmophloeum* is similar to that of commercial cinnamon species with lower coumarin content. The present study was aimed to investigate the antidyslipidemia effects of indigenous cinnamon (*Cinnamomum osmophloeum* Kaneh) leaf powder (CoLP) on hypercholesterolemia hamsters. Hyperlipidemia was induced by high-cholesterol (HChol) diet for 4 weeks. Two percent and 5% CoLP, and gemfibrozil (positive control; 0.25%) were administered for 10 weeks following HChol diet. Control groups were fed with normal diet (ND) or ND+5% CoLP. Behavioral, physiological, and serum biochemical parameters were determined. We found that oral administration of CoLP for 10 weeks significantly reduced the HChol-induced increase of total cholesterol (TC), triglyceride, and low-density lipoprotein levels in plasma of hamsters. In addition, HChol-induced elevation of serum glutamic oxaloacetic transaminase and glutamic pyruvic transaminase levels was significantly reversed by CoLP in a dose-dependent manner, whereas blood urea nitrogen and creatinine levels were unaffected. Further standard diagnostic tests support that consumption of CoLP did not show any behavioral and morphological changes in hamsters. Furthermore, chemical composition analysis revealed that two new flavanol glycosides, kaempferol-3-*O*-α-ʟ-rhamnopyranosyl-(1→2)-α-ʟ-arabinofuranosyl-7-*O*-α-ʟ-rhamnopyranoside (**4**) and kaempferol-3-*O*-β-ᴅ-apiofuranosyl-(1→2)-α-ʟ-arabinofuranoside (**5**) along with 4 known flavonoid glycosides were identified in leaves of *C. osmophloeum*. Taken together, these results concluded that CoLP possessed strong antidyslipidemic effects. Therefore, *C. osmophloeum* leaves could be a safe food supplement for treating hypercholesterolemia.

Cinnamon is one of the most popular spices used around the world and it is the second most valuable spice (next to black pepper) sold in the US and European markets. According to the permits by The United States Federal Food, Drug, and Cosmetic Act, the term of cinnamon is including Ceylon cinnamon (*Cinnamomum verum* Berchthold and Presl.), Chinese cassia (*C. cassia* (L.) Berchthold and Presl.), and Indonesian cassia (*C. burmannii* C. G. Th. Nees). Although people have utilized cinnamon for a long time, the hepatotoxic compound, coumarin, was found cinnamon with various amounts.[1] For example, the level of coumarin in *Ceylon cinnamon* leaves was detected for up to 190 mg/kg, while the concentrations in *Cassia cinnamon* ranged from 700 to

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12,200 mg/kg.[2] Coumarin as a food flavoring agent has been banned since the 1950s, due to the hepatotoxicity found in the laboratory animals that consumed coumarin daily.[3] Therefore, for safety concerns, harvesting the cinnamon with low coumarin content becomes an important issue in the global spice market.

Indigenous cinnamon, *Cinnamomum osmophloeum* Kaneh, is a unique cinnamon tree grown in Taiwan. The most distinguished feature of *C. osmophloeum* is that the composition of leaf essential oil is similar to those of *C. cassia* bark essential oil.[4,5] A recent study by Yeh et al demonstrated that coumarin content in various clones of *C. osmophloeum* were much lower than that of *Cassia cinnamons*. In addition, *C. osmophloeum* leaf essential oils contain about 80% (w/w) of cinnamaldehyde and 0.4% to 2.7% (w/w) of eugenol[6] and the cinnamaldehyde-rich indigenous cinnamon could be a better candidate to produce essential oil with a longer storage time without decomposition compared to other commercial cinnamons. Thus, the leaves of *C. osmophloeum* could be a potential resource for a safer cinnamon substitute. In regard to the bioactivity investigation, a number of studies have demonstrated that *C. osmophloeum* leaf essential oils has strong insecticidal, larvicidal, and antimicrobial properties.[4,7-10]

In addition to the antimicrobial properties, the potential application of *C. osmophloeum* leaves in food supplements is an exciting subject. We have previously reported that *C. osmophloeum* leaf essential oils and cinnamaldehyde, a predominant compound, exerted xanthine oxidase inhibition and reduced serum uric acid levels in oxonate-induced mice.[5] Except for essential oils, oral administration of the hot water leaf extracts of *C. osmophloeum* reduced the serum levels of total cholesterol (TC), triglyceride (TG), and low-density lipoprotein cholesterol (LDL-C) in hyperlipidemic hamsters induced by high-fat diet.[11] However, the biological activities of raw indigenous cinnamon leaf powder were poorly elucidated. In the present study, we focus on the ground *C. osmophloeum* leaf powder (CoLP) as a food supplement and tend to investigate its hypolipidemic activity in high-cholesterol-fed hamsters.

As shown in Table 1, oral administration of 2% and 5% of CoLP in the presence or absence of high-cholesterol (HChol) diet for 10 weeks did not affect daily food intake and body weight gain in hamsters. The body weight gain of control group was comparatively slower than that of HChol and sample treatment groups. The increase of body weight over the treatment period could have resulted from animal growth and cholesterol consumption. However, the body weight increase in HChol and HChol with sample groups were statistically not significant. In addition, a gradual increase of food intake was observed in the control group along with the age, and the similar trend was found in all the treatment groups. Moreover, during the experimental period we did not find any physiological or morphological changes in either CoLP- or HChol diet-fed hamsters.

High levels of TC, TG, and LDL-C and by the lowered level of high-density lipoprotein cholesterol (HDL-C) in serum are characterized as dyslipidemia. The relationship between dyslipidemia and atherosclerosis has been an area of active research as the prevalence of coronary heart disease increases all over the world. In the present study, we found that the plasma levels of TC, TG, HDL-C, and LDL-C were significantly increased in HChol challenged group as compared to the control group. Treatment with CoLP significantly reduced the elevated levels of serum TC, TG, HDL-C, and LDL-C in experimental hamsters. As shown in Figure 1(a), oral administration of 5% CoLP significantly decreased plasma TC from 240 ± 11.2 to 186 ± 11.3 mg/dL, whereas treatment with 2% of CoLP does not exhibit any significant reduction in serum TC. The oral consumption of original HChol diet varied slightly among CoLP-administrated group because the leaf powder mixed in HChol diet, which accounted for the partial volume of daily intake. However, the bioactivity of CoLP in the reduction of plasma TC is still reliable because the levels of TC suppression were much significant than the bias of the amount of cholesterol

Table 1. Effects of CoLP on Daily Food Intake and Body Weight Gain in HChol Diet-Fed Hamsters.

Group	Body weight (g)			Food intake (g/day)		
	Week 0	Week 5	Week 10	Week 1	Week 5	Week 10
Control	98.1 ± 10.2	119 ± 7.9	116 ± 14.7	7.87 ± 0.63	7.98 ± 0.59	8.26 ± 0.61
HChol	97.7 ± 13.8	131 ± 15.6	139 ± 13.0	7.88 ± 0.76	8 ± 0.72	8.28 ± 0.87
Hchol + CoLP (2%)	97.7 ± 4.7	129 ± 11.2	132 ± 5.3	7.82 ± 0.54	7.97 ± 0.55	8.23 ± 0.55
Hchol + CoLP (5%)	98.8 ± 7.2	121 ± 11.2	128 ± 8.6	7.8 ± 0.62	8.02 ± 0.68	8.25 ± 0.63
Hchol + Gem (0.25%)	98.3 ± 7.6	121 ± 7.7	126 ± 7.2	7.85 ± 0.7	7.93 ± 0.71	8.28 ± 0.72
CoLP (5%)	97.2 ± 110.7	119 ± 11.7	129 ± 18.0	7.82 ± 0.65	7.96 ± 0.56	8.26 ± 0.71

HChol, high-cholesterol; CoLP, *Cinnamomum osmophloeum* leaf powder; Gem, gemfibrozil.
Hamsters fed with normal diet (control) or high-cholesterol (2% HChol) diet along with different doses of CoLP (2% and 5% of CoLP) or 0.25% Gem for 10 weeks. The changes in body weight gain and daily food intake were monitored once in every week for 10 weeks. Data are presented as mean ± SD (n = 8) value obtained in 0/1, 5, and 10 weeks.
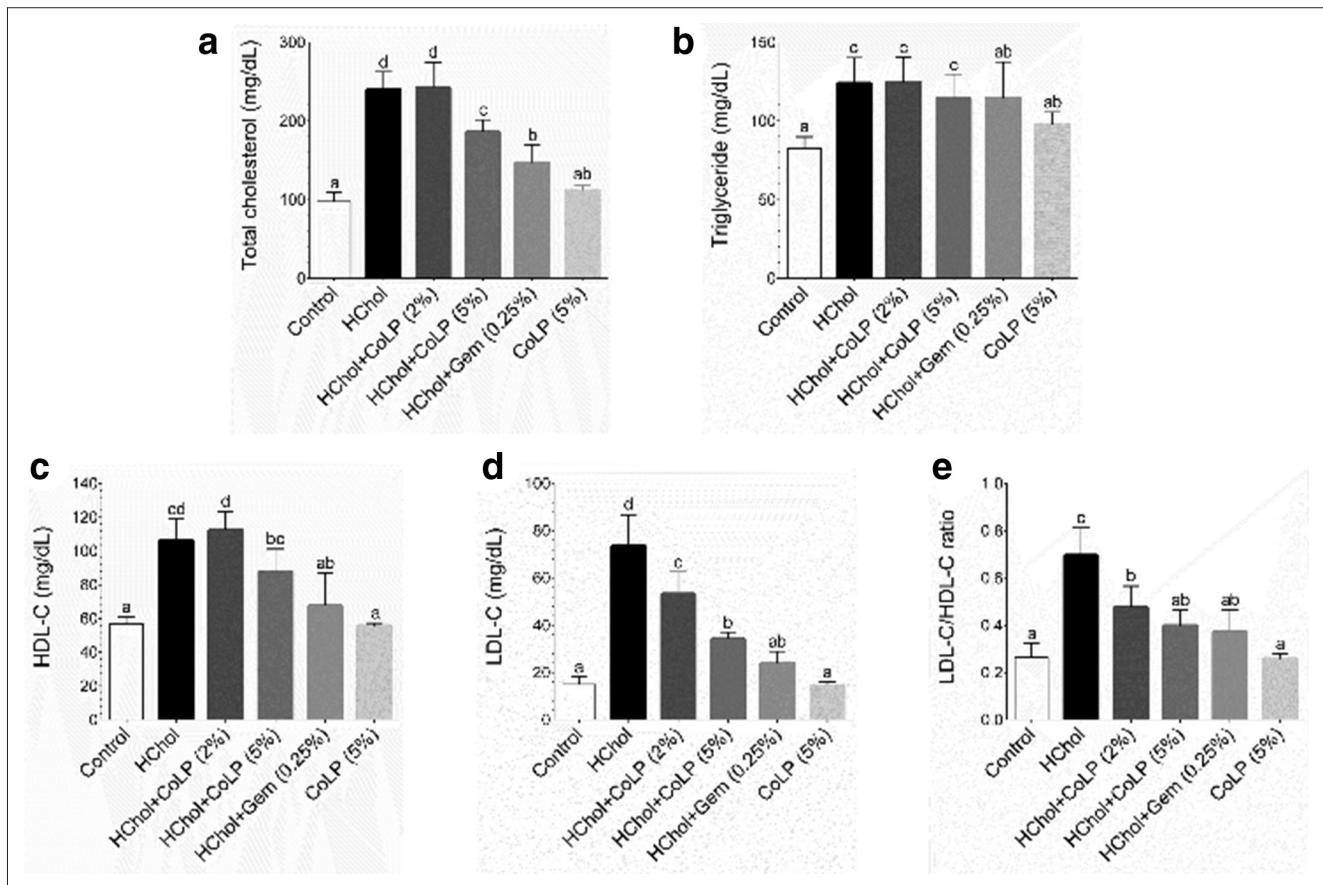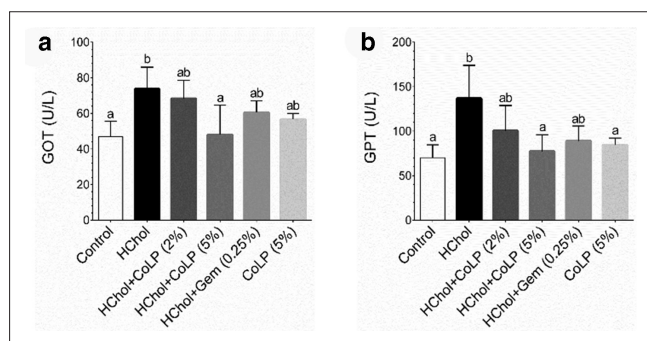
Figure 1. Effects of CoLP on serum lipid levels in HChol diet-induced hamsters. Hamsters fed with normal diet (control) or 2% HChol diet along with different doses of CoLP (2% and 5% of CoLP) or 0.25% Gem for 10 weeks. Total cholesterol (a), triglyceride (b), LDL-C (c), HDL-C (d), and LDL-C/HDL-C ratio (**e**) were determined in the blood serum. Data are presented as mean ± SD (*n* = 8). Mean values within each column with different labels (a, b, c, and d) are significantly different (*P* < 0.05). CoLP: *Cinnamomum osmophloeum* leaf powder; Gem: gemfibrozil; HChol: high-cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol.

consumption between HChol and HChol+CoLP (5%) groups. In addition, treatment with 5% of CoLP slightly increased TC levels from 97.8 ± 11.9 (control) to 111.7 ± 14.5 mg/dL. The increase of serum TC by 5% CoLP was not statistically significant. Meanwhile, an elevated plasma TG levels were observed in HChol diet-challenged hamsters, whereas treatment with 5% of CoLP slightly decreased plasma TG without statistical significance (Figure 1b). A similar pattern of result was observed in 0.25% gemfibrozil (Gem) treatment group.

It is well established that a lower level of LDL-C and a higher level of HDL-C in circulation are beneficial to the human health and could reduce the risk of cardiovascular disease. Therefore, determining the levels of HDL-C and LDL-C in serum is inevitable parameters of antidyslipidemia studies. In this study, we found that the LDL-C level was remarkably increased in HChol diet-fed hamsters (73.5 ± 13.3 mg/dL) compared to the control group (15.0 ± 3.0

mg/dL). However, treatment with 2% and 5% CoLP significantly reduced the HChol diet-induced LDL-C levels to 53.1 ± 9.4 and 34.0 ± 3.1 mg/dL, respectively (Figure 1c). It is noteworthy that hamsters exposed to 5% CoLP neither induce nor inhibit serum LDL-C and remained as basal level. On the other hand, we found that HDL-C levels were significantly higher in all HChol diet-challenged groups. As shown in Figure 1(d), serum HDL-C was significantly increased to 106.2 ± 12.7, 112.3 ± 11.2, 87.3 ± 13.7, and 67.4 ± 19.8 mg/dL in HCol, HChol+CoLP (2%), HChol+CoLP (5%), and HChol+Gem (0.25%) treatment groups, respectively, because both HDL-C and LDL-C levels are typical cholesterols, which are increased by a HChol diet. Concomitant with serum LDL-C level, exposure of hamsters to 5% of CoLP did not show any effects on serum HDL-C (Figure 1d). Next, we found a significant increase in the ratio of LDL-C/HDL-C in HChol diet-fed hamsters (0.69 ± 0.12) when compared to the control group (0.27 ±
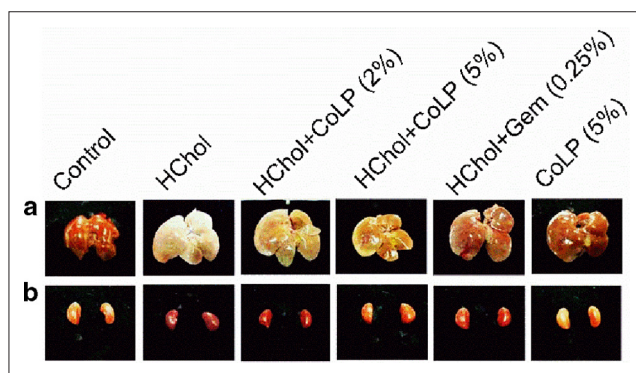
Figure 2. Effects of CoLP on serum hepatic enzyme levels in HChol diet-induced hamsters. Hamsters fed with normal diet (control) or 2% HChol diet along with different doses of 2% and 5% of CoLP or 0.25% Gem for 10 weeks. The levels of GOT (a) and GPT (b) were determined in the blood serum. Data are presented as mean ± SD ($n = 8$). Mean values within each column with different labels (a and b) are significantly different ($P < 0.05$). CoLP: *Cinnamomum osmophloeum* leaf powder; Gem: gemfibrozil; GOT: glutamic oxaloacetic transaminase; GPT: glutamic pyruvic transaminase; HChol: high-cholesterol.

Figure 3. Effects of CoLP on organs morphology in HChol diet-induced hamsters. Hamsters fed with normal diet (control) or 2% HChol diet along with different doses of CoLP (2% and 5% of CoLP) or 0.25% Gem for 10 weeks. The morphological changes in liver (a) and kidney (b) were examined. Each animal's liver and kidney was analyzed ($n = 8$) and 1 representative photomicrograph is shown here. CoLP: *Cinnamomum osmophloeum* leaf powder; Gem: gemfibrozil; HChol: high-cholesterol

0.06). In agreement with the LDL-C-lowering effect of CoLP, the LDL-C/HDL-C ratio was significantly decreased to 0.48 ± 0.09 and 0.40 ± 0.06 by 2% and 5% of CoLP, respectively (Figure 1e). In addition, when compared with the control group (0.27 ± 0.06), hamster fed with 5% of CoLP showed a similar ratio of LDL-C/HDL-C (0.26 ± 0.03). These data indicate that CoLP has strong hypolipidemic properties.

The hepatotoxic compound coumarin was found in many cinnamon species.[1,12] Despite that, the coumarin content in leaves of *C. osmophloeum* was much lower than that of Chinese cinnamon *Cassia cinnamons*.[6] Although the coumarin content was comparatively low in leaves of *C. osmophloeum*, we sought to examine whether the coumarin in CoLP could cause hepatotoxity in hamsters. Serum glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) levels in serum were considered as an important biomarker of liver damage. As shown in Figure 2(a), compared to the control group (47.0 ± 8.6 U/L), a remarkable increase of serum GOT was observed in HChol diet-fed group (73.7 ± 12.3 U/L), whereas treatment with 5% of CoLP significantly reduced the HChol-induced GOT level to 48.0 ± 16.5 U/L. On the other hand, elevated level of GPT (136.7 ± 37.3 U/L) was found in HChol diet-challenged group, whereas treatment with 5% CoLP significantly reduced HChol-induced GPT level to 77.1 ± 18.8 U/L (Figure 2b). Of note, the HChol-induced GOT and GPT levels were decreased by 2% CoLP and 0.25% Gem without statistical significance. Furthermore, hamsters exposed to 5% GoLP slightly increased GOT and GPT levels in the blood stream. However, the increase was not statistically significant. In addition, we found that neither CoLP nor HChol

diet affected the plasma levels of glucose, amylase and lipase in experimental hamsters (Supplementary Figure S1).

The increase in risk of advanced kidney failure is associated with various factors including lipid abnormalities. A recent study demonstrated that short-term HChol diet accelerates renal injury in hamsters as indicated by increased levels of blood urea nitrogen (BUN) and creatinine.[13] In contrast, in the present study we did not find any liver injury caused by either HChol or CoLP diet for 10 weeks as evidenced by no alteration being in plasma levels of BUN and creatinine (Supplementary Figure S2). To determine the pathological effects of HChol and CoLP, we examined the color and surface morphology of liver and kidney in CoLP diet-fed hamsters in the presence or absence of HChol diet. As shown in Figure 3(a), none of the hamsters in the normal diet group developed obvious pathological changes in the liver; the liver color was red-brown and the surface morphology was normal at the end of the experiment, whereas obvious pathological abnormalities were found in the HChol diet group, indicating that the liver color was changed to yellow-brown and lipid accumulation was noted. However, following treatment with CoLP at 2 doses, the liver color and surface morphology were greatly improved compared with HChol diet-only-fed hamsters. Of note, hamsters fed with 5% CoLP for 10 weeks did not show any detectable changes in liver color and surface morphology. Next we found that either CoLP or HChol diet did not modulate the color and surface morphology of hamsters during the experimental period (Figure 3b). Taken together, the presented data strongly support that CoLP could be a promising food supplement to lower the blood cholesterol.

Compounds **1** to **6** were isolated from hot water extracts of *C. osmophloeum* leaves. According to the analysis of their

spectroscopic data, 4 known compounds were identified, namely kaempferol-3-*O*-β-D-glucopyrano-syl-(1→4)-α-L-rhamnopyrano-syl-7-*O*-α-L-rhamnopyrano-side (**1**), kaempferol-3-*O*-β-D-apio-furanosyl-(1→2)-α-L-arabinofuranosyl-7-*O*-α-L-rhamnopyranoside (**2**), kaemp-feritrin (**3**), and kaempferol-3-*O*-α-L-rhamnopyranoside.[6,14] In addition to 4 known compounds, the structures of 2 new flavanol glycosides, kaempferol-3-*O*-α-L-rhamnopyrano-syl-(1→2)-α-L-arabinofuranosyl-7-*O*-α-L-rhamnopyrano-side (**4**) and kaempferol-3-*O*-β-D-apiofuranosyl-(1→2)-α-L-arabinofuranoside (**5**), were identified by spectral analyses. Compound **4** was isolated as a yellow solid. It showed UV maxima at λ_{max} 265 and 346 nm, which are typical for flavo-noid glycosides. Electrospray ionization mass spectrum (ESI-MS) of **4** had a strong peak at *m/z* 709 [M-H]⁻ in the negative mode, which is consistent with the molar mass of 710 g/mol. ESI-MS/MS showed fragments from subsequent loss of a deoxyhexose *m/z* 563 [M-147]⁻, loss of 2 sugar units *m/z* 430 [M-280]⁻, loss of 2 deoxyhexose *m/z* 417 [M-293]⁻, and finally a fragment at *m/z* 284 corresponding to the [M-2H]⁻ ion peak of kaempferol. ¹H and ¹H–¹H COSY nuclear magnetic resonance (NMR) data of **4** showed char-acteristic aromatic spin systems of rings A and B in kaemp-ferol. Signals of an AA'BB' system at δ_H 8.01 (d, *J* = 8.4 Hz) and 6.94 (d, *J* = 8.4 Hz), and each integrated for 2 protons, which were assigned to H-2′/H-6′ and H-3′/H-5′, respec-tively. In addition, 2 *meta* coupled protons at δ_H 6.48 (d, *J* = 1.8 Hz) and 6.77 (d, *J* = 1.8 Hz) were observed and assigned to H-6 and H-8, respectively. ¹³C NMR spectrum exhibited the presence of 3 anomeric carbons at δ_C 107.9, 101.3, and 99.8 ppm, and their corresponding protons were detected at δ_H 5.73 (br s), 4.97 (br s), and 5.57 (br s), respectively, in the heteronuclear multiple-quantum correlation (HMQC) spec-trum. Careful analysis of two-dimensional (2D) NMR data (correlation spectroscopy [COSY], HMQC, and heteronu-clear multiple bond correlation [HMBC]) revealed the pres-ence of 2 rhamnose- and one arabinose-sugar units, which agreed with the observed ESI-MS/MS fragments. The sequence of the sugar units and their attachment to the agly-cone moiety in **4** were confirmed by ESI-MS/MS, chemical shifts of the anomeric carbons and their protons, and HMBC correlations of the anomeric protons. The fragment observed at *m/z* 430 and the HMBC correlation of the anomeric proton at δ_H 5.73 ppm to C-3 (δ_C 135.2 ppm) indicated the attach-ment of the arabinose sugar to C-3 of the kaempferol agly-cone. The signal detected at *m/z* 563 as well as HMBC correlations of the anomeric proton at δ_H 5.57 ppm and the *meta*-coupled protons to C-7 (δ_C 163.6 ppm) indicated the attachment of the rhamnose unit to C-7. Finally, fragments observed at *m/z* 563 and 284, as well as the HMBC correla-tion of the anomeric proton at δ_H 4.97 ppm to C-2″ (δ_C 88.5 ppm) indicated the rhamnose-(1-2)-arabinose connection. This was confirmed by the characteristic downfield signal of C-2″ at δc 88.5 (Δδc 8 ppm). This was corroborated by the characteristic ¹³C NMR data which are in agreement with

those reported for α-L-rhamnopyranoside.[15] The pentose was identified as arabinofuranose based on the characteristic ¹H and ¹³C NMR signals (Supplementary Table S1), which are in accordance with the literature[16] and **2**. The observed broad singlet of the anomeric proton at 5.73 ppm is indica-tive of its equatorial orientation. The furanose nature of the α-L-arabinosyl unit was further confirmed by the observed downfield signal of C-4″ at δ_C 87.7 (Δδc 7 ppm) and the upfield signal of C-5″ at δ_C 62.4 (Δδc 5 ppm) compared with the reported data for arabinopyranosides[17,18] as well as by the ³*J*-HMBC correlation of its anomeric proton to C-4″ (δ_C 87.7 ppm). Finally, the rhamnopyranosyl unit was deduced from the characteristic ¹³C NMR signals, which agree with the data of **3**. The chemical shift of the anomeric proton of the rhamno-pyranosyl unit appearing at δ_H 4.97 ppm is typical for a terminal rhamnosyl unit. The spectroscopic data con-firmed the structure of **4** as kaempferol 3-α-L-rhamnopyra-nosyl-(1→2)-α-L-arabinofuranosyl-7-*O*-α-L-rhamnopyra-noside, which was a new compound. Each sugar unit was completely assigned based on 2D NMR data analysis and comparison with previously reported data. The spin system containing the anomeric proton at δ_H 5.57 ppm corresponded to a rhamnopyranosyl unit.

Compound **5** was isolated as a yellow solid. It showed UV maxima at λmax 265 and 347 nm. ESI-MS of **5** exhibited a strong peak at *m/z* 549 [M-H]⁻ in the negative mode, which is consistent with the molar mass of 550 g/mol. ESI-MS/MS showed fragments from subsequent loss of a pentose *m/z* 417 [M-132]⁻, and finally a fragment at *m/z* 285 ([M-H]⁻) corre-sponding to the ion peak of kaempferol. Careful comparison of NMR data as well as ESI-MS/MS data of **5** with those of **2** showed that **5** is almost the same with **2**, including kaemp-ferol aglycone, arabinosyl, and apiosyl sugar units. The only difference is the loss of a rhamnopyranoside subunit of **5**. Furthermore, the HMBC correlations of the anomeric proton at δ_H 5.19 ppm to C-2″ (δ_C 88.0 ppm) confirmed the apiose-(1-2)-arabinose connection. As well as the HMBC correla-tion of the anomeric proton (5.54 ppm) to C-3 (δ_C 134.8 ppm) established the attachment of arabinose sugar to C-3. Based on those evidences, a new compound, kaemp-ferol-3-*O*-β-D-apiofuranosyl-(1→2)-α-L-arabinofuranoside (**5**), was confirmed. A detailed compound identification and characterization are summarized in the Supplementary data section.

Previous studies have shown that kaemferol and kaem-ferol glycosides exhibited strong hypolipidemic effects by decreased levels of TGs and cholesterol, and reduced body weight gain in high-fat-diet-challenged experimental ani-mal models.[19,20] Therefore, we believe that kaempferol glu-cosides content in CoLP may exert its hypolipidemic effects. However, further studies are highly warranted to identify the major compound responsible for this effect. In the near future, we will examine the antidyslipidemia effects of isolated kaemferol glycosides *in* in vitro and in vivo models.

Moreover, we previously reported that hot water extract of leaves of *C. osmophloeum* reduced body weight gain and lipid levels in the circulation.[11] The present study signify the direct application (without extraction procedure) of leaves of *C. osmophloeum* to achieve an immediate anti-obesity or lipid-lowering effects.

Experimental

Plant Samples

The leaves of *C. osmophloeum* were collected in June 2015 from Nantou County, Taiwan, and was identified by Prof Yen-Hsueh Tseng, Department of Forestry, National Chung Hsing University. The voucher specimen was deposited in the herbarium of the same university. The leaves were washed, air-dried, and further ground into thin powder. The leaf powder was stored at −20°C until used. All chemicals and solvents used for the isolation and identification of phyto-compounds were high-performance liquid chromatography grade and supplied by either Merck (Darmstadt, Germany) or Sigma-Aldrich (St Louis, MO, USA). Deionized water was prepared using a Milli-Q water purification system (Millipore, MA, USA). All the solutions were filtered through 0.45 μm membranes (Schleicher & Schuell, Germany) and degassed by an ultrasonic bath before use. Gem with purity ≧99% was purchased from Sigma-Aldrich and used as a reference cholesterol-lowering drug. A detailed procedure for extraction and compound identification is summarized in the supplementary information.

Animal and Treatment

Seven-week-old healthy male Golden Syrian hamsters (100 ± 10 g) were purchased from BioLASCO, Taipei, Taiwan and were maintained in plastic caged housing separately in a specifically designed pathogen-free isolation facility with a 12-hour light and 12-hour dark cycle, 60% relative humidity with an optimum temperature of 25°C. The animals were given free access to regular rodent chow (LabDiet 5001 Rodent diet, Purina Mills LLC, St Louis, MO, USA) and water for 4 weeks. Animal experiments were designed and performed in accordance with the *Guidelines for the Care and Use of Laboratory Animals* of The Chinese Taipei Society for Laboratory Animal Science. After accommodated to the laboratory condition, 48 hamsters were weighed and randomly assigned into 6 groups of 8 animals each group. Groups I and VI served as control groups and received normal chow diet, while groups II to V received HChol diet for 4 weeks to induce hyperlipidemia condition. HChol diet was prepared by addition of 0.2% cholesterol (CAS# C8503, Sigma-Aldrich) to the regular rodent chow diet. After diet induction, each group was subjected to different treatments for 10 weeks as the following conditions. Animals that received normal diet throughout the experiment were considered as control group (group A). Animals that received

HChol diet only throughout the experiment were assigned as hyperlipidemic group (group B). Hamsters that were fed with HChol diet containing 2% of CoLP (group C, HChol+2% CoL), 5% of CoLP (group D, HChol+5% CoL), and 0.25% Gem (group E, HChol+0.25% Gem) were sample treatment groups. Animals fed with normal diet containing 5% CoLP were group F. At the end of the 10-week treatment, animals were anesthetized and killed by inhalation of 2% isoflurane. The blood samples were collected from the cardiac puncture and then the plasma samples were prepared by centrifugation for 10 minutes at 2000 × *g*. The plasma levels of TC, TG, HDL-C, LDL-C, GOT, GPT, BUN, creatinine, glucose, amylase, and lipase were measured. All analyses were carried out according to the spectrophotometry of Chiron Diagnostics Corporation (Oberlin, OH, USA) using the Express Plus Automatic Clinical Chemistry Analyzer (Chiron).

Statistical Analyses

Data were expressed as mean ± SD (n = 8). Differences in food intake, body weight gain, and serum biochemical parameter were analyzed by using one-way analysis of variance followed by Tukey's multiple comparisons test that was performed using GraphPad Prism version 7.0 (GraphPad Software Inc., La Jolla, CA, USA). Experimental groups not sharing a common letter were significantly different ($P < 0.05$).

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Supplemental Material

Supplemental material for this article is available online.

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