JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Chemical Composition and Hypoglycemic and Pancreas-Protective Effect of Leaf Essential Oil from Indigenous Cinnamon (*Cinnamomum osmophloeum* Kanehira)

Shih-Chieh Lee,[†,⊥] Wen-Xin Xu,[‡,⊥] Li-Yun Lin,[§] Jia-Jung Yang,[‡] and Cheng-Tzu Liu*[,‡,∥]

[†]Department of BioIndustry Technology, Da-Yeh University, Changhua 51591, Taiwan, Republic of China

[‡]School of Nutrition, Chung Shan Medical University, Taichung 40242, Taiwan, Republic of China

[§]Department of Food Science and Technology, Hungkuang University, Taichung 43302, Taiwan, Republic of China

[∥]Department of Nutrition, Chung Shan Medical University Hospital, Taichung 40242, Taiwan, Republic of China

ABSTRACT: The antidiabetic effect of cinnamon has generated broad interest during the past decade. We investigated the hypoglycemic activity and pancreas-protective effect of leaf essential oil from indigenous cinnamon (CO) in diabetic rats induced with streptozotocin (STZ, iv, 65 mg/(kg bw)) and found linalool to be the major component representing 40.24% of the CO composition. In diabetics, all tested doses of CO significantly lowered fasting blood glucose and fructosamine and are concomitant with elevated plasma and pancreatic insulin levels under a fasting condition. However, during the oral glucose tolerance test (OGTT) period the effect of 25 and 50 mg/(kg bw) of CO was shown to be less effective than that of 12.5 mg/(kg bw) in ameliorating the accumulation of plasma insulin. In addition, at 12.5 mg/(kg bw), CO significantly ameliorated pancreatic values of thiobarbituric acid reactive substances and activities of superoxide dismutase and glutathione reductase in diabetics to an extent greater than that of higher CO doses. At doses 12.5 and 25 but not 50 mg/(kg bw), CO significantly ameliorated pancreatic levels of interleukin-1β and nitric oxide. In conclusion, appropriate doses of CO of the linalool chemotype exhibited therapeutic potential in glycemic control in diabetes that was at least partially resulted from improved insulin secretion. The ameliorated oxidative stress and proinflammatory environment in the pancreas by CO may provide a protective effect on pancreatic β cells and warrant further investigation.

KEYWORDS: *Cinnamomum osmophloeum Kanehira, hypoglycemic effect, inflammation, leaf essential oil, oxidative stress, pancreas, streptozotocin*

INTRODUCTION

Diabetes mellitus is a metabolic disturbance that results from insufficient insulin secretion and insulin resistance with persistent hyperglycemia that eventually results in specific complications. Poorly controlled blood glucose in diabetes has been implicated in the progression of this disease. Both acute and chronic hyperglycemia have been shown to cause elevated concentrations of reactive oxygen species (ROS) and lowered enzymatic and nonenzymatic cell antioxidant defenses in normal and diabetic conditions.[1,2] Diabetes patients are reported to have lowered antioxidant capacity accompanied by elevated indices of oxidative stress.[1,3] Previous studies have suggested that hyperglycemia may be a proinflammatory state; elevated levels of proinflammatory cytokines, including tumor necrosis factor (TNF)-α and interleukin (IL)-1β, were found in the peripheral plasma of diabetic patients.[4] The production of ROS resulting from acute hyperglycemia subsequently leads to elevated peripheral levels of IL-1, TNF-α, and nitric oxide (NO) derivatives through activated transcription factors such as nuclear factor-κB or activator protein-1 in various cell types.[5]

Cinnamon is a small evergreen tree of the genus *Cinnamomum* that belongs to the *Lauraceae* family and encompasses approximately 250 species. Numerous species of the genus *Cinnamomum* are grown commercially worldwide and sold as cinnamon. The inner bark of trees from the genus *Cinnamomum* is also called cinnamon and commonly used as a spice in food to give taste, aroma, and flavor and to act as a preservative. Other folk uses of cinnamon include applying its essential oil as a fragrance in cosmetics, perfumes, or cigarettes. Cinnamon also has a long history of therapeutic use for various health problems including diabetes. However, it was not until the past decade that the possible antidiabetic role of cinnamon in humans and in experimental animals has been investigated scientifically.[6−8] The results of the antidiabetic effect of cinnamon are inconsistent. Although several clinical studies and a few meta-analyses have confirmed the usefulness of cinnamon as an antidiabetic agent,[7−9] the results of other clinical studies and meta-analysis have shown cinnamon to be ineffective in oral glucose tolerance, insulin sensitivity, fasting blood glucose, glycated hemoglobin, lipid profile, or peripheral insulin levels in type 2 diabetes patients.[10−12]

One problem with the research on the antidiabetic effect of cinnamon is that different species of cinnamon trees and various preparations have been used in various studies. In addition to such studies failing to state the species of cinnamon used, others studies provided the source of cinnamon among which *C. cassia*, *C. zeylanicum*, and *C. burmannii* are mostly used. Scant studies

Received: March 7, 2013
Revised: April 20, 2013
Accepted: April 30, 2013
Published: April 30, 2013

have shown certain cinnamon compositions/derivatives to possess effective hypoglycemic action. Ziegenfuss et al.[13] identified polyphenol type-A polymers as possible active chemicals in a human study, whereas Kim et al.[14] reported that naphthalene-methyl ester, a derivative of cinnamon composition dihydroxyhydrocinnamic acid, is an active compound in experimental animals. Several recent studies have focused on cinnamonaldehyde as an active antidiabetic constituent in cinnamon.[15,16]

Indigenous cinnamon (*C. osmophloeum*) cultivated in Taiwan has been classified into six chemotypes according to the dominant compounds in their leaf essential oil: cinnamaldehyde type, cinnamaldehyde/cinnamyl acetate type, cinnamyl acetate type, linalool type, camphor type, and mixed type according to gas chromatography−mass spectrometry (GC/MS) and cluster analyses of their leaf essential oils.[17] However, the evidence showing the antidiabetic effect of indigenous cinnamon is currently limited. We prepared a leaf essential oil from *C. osmophloeum*, analyzed its chemical composition, and compared its antidiabetic effects with that of cinnamaldehyde.

MATERIALS AND METHODS

Chemicals and Reagents. The authentic compounds for a leaf essential oil from indigenous cinnamon (CO) composition assay were purchased from Acros Organics (Geel, Belgium): benzaldehyde, L(−)-borneol, L-bornyl acetate, cineole, *p*-cymene, linalool, α-terpineol, terpinen-4-ol, α-pinene, (+)-limonene, neral, geraniol, eugenol, cinnamyl alcohol, and *trans*-cinnamaldehyde. The cinnamaldehyde congeners, including cinnamic acid, cinnamyl acetate, 4-hydroxybenzaldehyde, and 3-phenylpropionaldehyde, were also obtained from Acros Organics (Geel, Belgium). All were of reagent grade. Phenylmethylsulfonyl fluoride (PMSF) was purchased from Roche (Indianapolis, IN); the rat IL-1β enzyme-linked immunosorbent assay (ELISA) kit and the rat TNF-α ELISA kit were purchased from eBioscience (San Diego, CA); the Bio-Rad protein assay kits were purchased from Bio-Rad Laboratories (Richmond, CA). The glucagon-like peptide-1 (GLP-1) ELISA and gastric inhibitory peptide (GIP) ELISA kits were purchased from Merk Millipore (Darmstadt, Germany); the glycated serum protein enzyme assay kit was purchased from Diazyme (Poway, CA); the rat insulin ELISA kit was purchased from Mercodia (Uppsala, Sweden); the nitrite/nitrate kit and superoxide dismutase (SOD) assay kit were purchased from Cayman (Ann Arbor, MI). Streptozotocin and all other chemicals were purchased from Sigma Chemical Company (St. Louis, MO).

Plant Materials and Isolation of Essential Oil. Fresh *C. osmophloeum* Kaneh leaves were collected from a research farm at National Chiayi University, in Shekou, a central Taiwan county. The leaves were collected in May of 2009 and were subjected to hydrodistillation in an 80 L steam-distillator for 2 h, a pilot-scale-modified Clevenger-type apparatus, after which the oil contents were identified. Leaf essential oils were stored in airtight containers in the dark at −20 °C prior to analysis by GC/MS.

Chemical Characterization of Essential Oil. The HP6890 series gas chromatograph coupled with a 5973 network mass selective detector was used to quantify and determine the chemical components in the essential oils of the *C. osmophloeum* leaves. The authentic compounds include benzaldehyde, L(−)-borneol, L-bornyl acetate, cineole, *p*-cymene, linalool, α-terpineol, terpinen-4-ol, α-pinene, (+)-limonene, neral, geraniol, eugenol, cinnamyl alcohol, and *trans*-cinnamaldehyde. The cinnamaldehyde congeners, including cinnamic acid, cinnamyl acetate, 4-hydroxybenzaldehyde, and 3-phenylpropionaldehyde, were all reagent grade.

For GC/MS sample analysis, a capillary column-type DB-1 (i.d. 0.25 mm × 60 m; membrane thickness, 0.25 μm) was used. Helium was used as the carrier gas and was operated at a flow rate of 1 mL/min. The ionization potential used was 70 eV. The temperature of the ion source was set at 230 °C. The flux ratio was set at 50:1. The initial temperature was set at 40 °C for 10 min, programmed at 2 °C/min up to 240 °C, and held at this temperature for 20 min.

Aliquots (0.5 μL) of the essential oils were measured with a GC microsyringe and analyzed with GC/MS. Quantification of each constituent in the essential oil was calculated from the integrated diagrams obtained by eq 1:

$$Q = A \times Y \quad (1)$$

where Q is the quantity of each volatile constituent in essential oil, A is the percent peak area in the gas chromatograms occupied by each constituent, and Y is the recovery yield of essential oils.

A reference mixture of *n*-alkanes (C5−C25) was used to calculate the retention indices (RI) from the retention time (t_R) for each component. By referring to the documented data, each exact constituent was deduced. Alternatively, by comparing the GC/MSD spectra, each component was qualitatively searched by mapping and confirmed. For volatile structural analysis, we referred to the database provided by Schonburg and Dielmann,[18] Wiley MS Chemstation Libraries, NBS Computer Data Base, and authentic cited patterns.

Animals and Treatments. Four week old weanling male Wistar rats were purchased from the National Animal Breeding and Research Center (Taipei, Taiwan). The animals were kept under a 12 h light−dark cycle at an ambient temperature of 23 °C and were given free access to water and standard rat feed (Rodent Diet 5001; Purina Mills, Richmond, IN). All animals were allowed to adapt to the environment for one week after their arrival before beginning the experiment. Diabetes was induced by injecting streptozotocin (iv, 65 mg/(kg bw)), and the control rats were injected with the same vehicle volume as described by Liu et al.[19] Three days after injection, the diabetic animals were randomly assigned to six groups and received by gavage leaf essential oil of *C. osmophloeum* (CO; 12.5, 25, or 50 mg/(kg bw)), cinnamaldehyde (40 mg/(kg bw)), glibenclamide (1.2 mg/(kg bw)), or the vehicle (corn oil; 2 mL/(kg bw)) every other day for three weeks. According to our preliminary study, the CO doses and treatment duration used in our study do not affect the metabolic characteristics in normal rats, which include growth rate, food and water intake, and urine excretion. The control rats received corn oil by gavage (2 mL/(kg bw)). During the three weeks of treatment, the animals were housed in metabolic cages and were given free access to water and a powdered diet (Rat Diet 5012; Purina Mills, Richmond, IN). Food and water intakes and urine excretion were measured.

The rats received an oral glucose tolerance test on day 17 and were killed on day 21, as described by Liu et al.[19] All plasma samples obtained were stored at −20 °C until analyzed. Measurements of insulin, fructosamine, and nonesterified fatty acids in plasma were conducted within two weeks. Housing conditions and experimental procedures were performed according to the NIH Guide for the Care and Use of Laboratory Animals, and all protocols were approved by the ethical committee for animal experimentation at Chung Shan Medical University, Taichung, Taiwan.

Oral Glucose Tolerance Test. The oral glucose tolerance test was performed by orally administering gavage by a solution of 10% (w/v) glucose (1 g/(kg bw)). Blood samples were drawn from the lateral tail vein immediately before and 5, 10, 15, 30, 60, 90, 120, and 180 min after bolus glucose loading. Heparin-containing blood samples were immediately centrifuged, and the plasma was separated and frozen at −20 °C until analyzed for glucose, insulin, GLP-1, and GIP. The area under the concentration-by-time curve of glucose, insulin, GLP-1, and GIP during the oral glucose tolerance test was calculated.

Biochemical Analysis of Blood Samples. For glucose analysis, plasma was deproteinized, and glucose concentrations analyzed enzymatically.[19] Plasma concentrations of insulin, fructosamine, GLP-1, and GIP were determined spectrophotometrically with a rat insulin ELISA kit, a fructosamine kit, a rat GLP-1 ELISA kit, and a rat GIP ELISA kit, respectively, according to manufacturer instructions.

Biochemical Analysis of Pancreas Samples. Immediately following removal of the pancreas, the organ was irrigated with cold phosphate-buffered saline (PBS) (pH 7.2) containing 1 mM phenylmethylsulfonyl fluoride to inhibit protease activity, followed by storing

Table 1. Volatile Components in the Native Leaf Essential Oils from *C. osmophloeum* Kaneh.[a]

no.	compd	RI[a]	composition (%)	CAS No.	formula	MW	no.	compd	RI	composition (%)	CAS No.	formula	MW
1	ethenylbenzene[b]	877	0.01%	100-42-5	C_8H_8	104	52	(*cis, trans*)-α-farnesene	1434	0.20%	26560-14-5	$C_{15}H_{24}$	204
2	tricyclene	920	0.01%	508-32-7	$C_{10}H_{16}$	136	53	aromadendrene	1439	0.23%	489-39-4	$C_{15}H_{24}$	204
3	α-thujene	924	0.06%	2867-05-2	$C_{10}H_{16}$	136	54	β-farnesene	1450	0.06%	18794-84-8	$C_{15}H_{24}$	204
4	α-(+)-pinene	932	1.38%	80-56-8	$C_{10}H_{16}$	136	55	α-humulene	1452	0.37%	6753-98-6	$C_{15}H_{24}$	204
5	benzaldehyde	936	0.11%	100-52-7	C_7H_6O	106	56	α-acoradiene	1457	0.06%	24048-44-0	$C_{15}H_{24}$	204
6	α-fenchene	943	0.03%	471-84-1	$C_{10}H_{16}$	136	57	alloaromadendrene	1459	0.15%	999209-01-5	$C_{15}H_{24}$	204
7	camphene	945	0.63%	79-92-5	$C_{10}H_{16}$	136	58	α-amorphene	1472	0.21%	483-75-0	$C_{15}H_{24}$	204
8	sabinene	967	0.08%	3387-41-5	$C_{10}H_{16}$	136	59	α-curcumene	1474	0.22%	644-30-4	$C_{15}H_{22}$	202
9	(−)-β-pinene	972	0.45%	18172-67-3	$C_{10}H_{16}$	136	60	germacrene-d	1477	0.09%	23986-74-5	$C_{15}H_{24}$	204
10	β-myrcene	984	0.35%	123-35-3	$C_{10}H_{16}$	136	61	β-selinene	1483	0.07%	17066-67-0	$C_{15}H_{24}$	204
11	α-phellandrene	997	0.04%	99-83-2	$C_{10}H_{16}$	136	62	γ-muurolene	1486	0.02%	30021-74-0	$C_{15}H_{24}$	204
12	3-carene	1004	0.01%	13466-78-9	$C_{10}H_{16}$	136	63	α-zingibirene	1488	0.02%	495-60-3	$C_{15}H_{24}$	204
13	α-terpinene	1010	0.03%	99-86-5	$C_{10}H_{16}$	136	64	β-patchoulene	1491	0.27%	514-51-2	$C_{15}H_{24}$	204
14	cymene	1015	0.68%	25155-15-1	$C_{10}H_{14}$	134	65	acetyleugenol	1494	0.51%	93-28-7	$C_{12}H_{14}O_3$	206
15	2-hydroxybenzaldehyde	1019	0.14%	90-02-8	$C_7H_6O_2$	122	66	β-bisabolene	1502	0.21%	495-61-4	$C_{15}H_{24}$	204
16	1,8-cineol	1021	0.64%	470-82-6	$C_{10}H_{18}O$	154	67	γ-muurolene	1508	0.36%	30021-74-0	$C_{15}H_{24}$	204
17	limonene	1024	1.53%	138-86-3	$C_{10}H_{16}$	136	68	1*S*, *cis*-calamenene	1513	0.16%	483-77-2	$C_{15}H_{22}$	202
18	*trans*-α-ocimene	1030	0.05%	3779-61-1	$C_{10}H_{16}$	136	69	δ-cadinene	1517	0.96%	483-76-1	$C_{15}H_{24}$	204
19	β-ocimene	1041	0.05%	13877-91-3	$C_{10}H_{16}$	136	70	cadina-1,4-diene	1528	0.04%	29837-12-5	$C_{15}H_{24}$	204
20	γ-terpinene	1052	0.07%	99-85-4	$C_{10}H_{16}$	136	71	α-calacorene	1533	0.13%	999343-25-8	$C_{15}H_{20}$	200
21	*cis*-linalool oxide	1063	0.13%	999083-25-3	$C_{10}H_{18}O_3$	170	72	(+)-nerolidol	1556	0.48%	142-50-7	$C_{15}H_{26}O$	222
22	*trans*-linalool oxide	1077	0.13%	11063-78-8	$C_{10}H_{18}O_2$	170	73	lauric acid	1566	0.07%	143-07-7	$C_{12}H_{24}O_2$	200
23	terpinolene	1081	0.10%	586-62-9	$C_{10}H_{16}$	136	74	(+) spathulenol	1572	0.39%	77171-55-2	$C_{15}H_{24}O$	220
24	linalool	1102	40.24%	78-70-6	$C_{10}H_{18}O$	154	75	caryophyllene oxide	1575	1.00%	1139-30-6	$C_{15}H_{24}O$	220
25	camphor	1131	9.38%	464-49-3	$C_{10}H_{16}O$	152	76	(+)-ledol	1581	0.07%	577-27-5	$C_{15}H_{26}O$	222
26	benzylacetaldehyde	1135	0.22%	104-53-0	$C_9H_{10}O$	134	77	guaiol	1592	0.05%	489-86-1	$C_{15}H_{26}O$	222
27	3-phenyl-2-propenal	1158	4.06%	104-55-2	C_9H_8O	132	78	humulene oxide II	1599	0.13%	19888-34-7	$C_{15}H_{24}O$	220
28	4-terpineol	1169	0.20%	562-74-3	$C_{10}H_8O$	154	79	alloaramadendrene oxide (I)	1617	0.01%	999012-25-8	$C_{15}H_{24}O$	220
29	estragole	1181	1.31%	140-67-0	$C_{10}H_{12}O$	148	80	ledene oxide (II)	1619	0.08%	999029-51-9	$C_{15}H_{24}O$	220
30	*trans*-cinnamaldehyde	1190	0.17%	14371-10-0	C_9H_8O	132	81	6-cadinol	1621	0.06%	19435-97-3	$C_{15}H_{26}O$	222
31	octyl acetate	1195	0.05%	112-14-1	$C_{10}H_{20}O_2$	172	82	10,10-dimethyl-2,6-dimethylene-bicyclo[7.2.0] undecan-5-ol	1626	0.03%	19431-80-	$C_{15}H_{24}O$	220
32	*cis*-citral	1219	0.07%	106-26-3	$C_{10}H_{16}O$	152							
33	cinnamaldehyde	1249	6.87%	104-55-2	C_9H_8O	132	83	isoaromadendrene epoxide	1630	0.03%	999012-24-0	$C_{15}H_{24}O$	220
34	chavicol	1261	0.25%	501-92-8	$C_9H_{10}O$	134	84	τ-cadinol	1635	0.03%	5937-11-1	$C_{15}H_{26}O$	222
35	*trans*-anethol	1270	0.04%	4180-23-8	$C_{10}H_{12}O$	148	85	*o*-methoxy cinnamyl acetate	1639	0.08%		$C_{12}H_{14}O_3$	206
36	bornyl acetate	1273	1.72%	76-49-3	$C_{12}H_{20}O_2$	196	86	α-cadinol	1647	0.19%	481-34-5	$C_{15}H_{26}O$	222
37	cinnamyl alcohol	1291	0.14%	104-54-1	$C_9H_{10}O$	134	87	methoxyeugenol	1675	0.10%	6627-88-9	$C_{11}H_{14}O_3$	194
38	eugenol	1340	0.55%	97-53-0	$C_{10}H_{12}O_2$	164	88	zerumbone	1713	0.03%	471-05-6	$C_{15}H_{22}O$	218
39	*cis*-cinnamic acid	1347	0.23%	102-94-3	$C_9H_8O_2$	148	89	farnesyl acetate	1821	0.03%		$C_{17}H_{28}O_2$	264
40	α-cubebene	1349	0.11%	17699-14-8	$C_{15}H_{24}$	204	90	6,10,14-trimethylpentadecan-2-one	1835	0.08%	502-69-2	$C_{18}H_{36}O$	268
41	geraniol acetate	1365	0.43%	105-87-3	$C_{12}H_{20}O_2$	196	91	farnesol	1897	0.03%	4602-84-0	$C_{15}H_{26}O$	222

dx.doi.org/10.1021/jf401039z | J. Agric. Food Chem. 2013, 61, 4905−4913

Table 1. continued

no.	compd	RI[a]	composition (%)	CAS No.	formula	MW	no.	compd	RI	composition (%)	CAS No.	formula	MW
42	(+)-cyclosativene	1368	0.05%	999209-12-9	$C_{15}H_{24}$	204	92	rimuene	1929	0.78%	1686-67-5	$C_{20}H_{32}$	272
43	α-ylangene	1371	0.09%	14912-44-8	$C_{15}H_{24}$	204	93	ent-pimara-8(14),15-diene	1946	0.10%	19882-10-1	$C_{20}H_{32}$	272
44	isoledene	1373	0.02%	999350-67-0	$C_{15}H_{24}$	204	94	hexadecanoic acid	1963	0.69%	57-10-3	$C_{16}H_{32}O_2$	256
45	copaene	1376	0.85%	3856-25-5	$C_{15}H_{24}$	204	95	bornyl cinnamate 1	1989	0.07%	6330-67-2	$C_{19}H_{24}O_2$	284
46	α-bourbonene	1383	0.07%	999137-43-5	$C_{15}H_{24}$	204	96	manoyl oxide	2009	0.14%	596-84-9	$C_{20}H_{34}O$	290
47	β-cubebene	1386	0.04%	13744-15-5	$C_{15}H_{24}$	204	97	(−)-kaurene	2038	0.10%	562-28-7	$C_{20}H_{32}$	272
48	β-elemene	1388	0.04%	515-13-9	$C_{15}H_{24}$	204	98	phytol	2106	0.03%	150-86-7	$C_{20}H_{40}O$	296
49	coumarin	1410	2.13%	91-64-5	$C_9H_6O_2$	146	99	linolenic acid	2128	0.17%	463-40-1	$C_{18}H_{30}O_2$	278
50	caryophyllene	1418	2.65%	87-44-5	$C_{15}H_{24}$	204	100	oleic acid	2134	0.12%	112-80-1	$C_{18}H_{34}O_2$	282
51	*trans*-cinnamyl acetate	1427	11.71%	21040-45-9	$C_{11}H_{12}O_2$	176	101	octadecanoic acid	2161	0.06%	57-11-4	$C_{18}H_{36}O_2$	284

[a]RI, Kovat's GC retention index calculated with C5−C25 *n*-alkanes as references. [b]These volatiles were identified by comparing with the standard or authentic sample, whereas others were identified by referring to the computer mass libraries to compare the mass spectrum with the published data of known chemical structures.

at −80 °C until assayed for insulin, oxidative stress, and inflammatory condition.

Insulin content was analyzed spectrophotometrically using the rat insulin ELISA kit according to manufacturer instructions. Lipid peroxidation was determined by measuring thiobarbituric acid reactive substances (TBARS) using a fluorescence spectrophotometer (Hitachi F4500, Tokyo, Japan), and the total glutathione (GSH) level was assessed spectrophotometrically by the GSH reductase−DTNB (5.5 dithiobis-2-nitrobenzoic acid) recycling procedure with a microplate reader (VersaMax; Molecular Devices Ltd., Sunnyvale, CA), conducted as previously described.[25] GSH peroxidase and GSH reductase were determined spectrophotometrically as previously described.[20] SOD activity was determined with the Randox SOD kit according to manufacturer instructions (Randox Laboratories Ltd., Antrim, UK) and was calculated by the degree of inhibition of 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride reaction with superoxide anion generated by xanthine-xanthine oxidase. The contents of TNF-α and IL-1β or nitrate/nitrite were measured spectrophotometrically using the rat TNF-α and IL-1β ELISA kits (Biosource International, Inc., Camarillo, CA) or the nitrate/nitrite kit (Roche Diagnostics, Mannheim, Germany), respectively, according to manufacturer instructions.

Statistical Analysis. The data are expressed as means ± standard deviation (SD) and were analyzed by one-way analysis of variance (ANOVA). Student's *t* test was used to detect differences in means between the control group and the group of diabetic rats. The Duncan multiple-comparison test was used to detect differences among the means of the streptozotocin-injected groups. A p value of less than 0.05 was considered significant. All statistical analyses were performed with commercially available software (SPSS 12 for Windows; SPSS Inc., Chicago, IL).

RESULTS

Volatile Components in Native Leaf Essential Oils from *C. osmophloeum* Kaneh. GC and GC/MS analyses indicated 101 volatile compounds present in leaf essential oil from *C. osmophloeum* (CO), including monoterpenoids, sesquiterpenoids, alcohols, phenols, aldehydes, ketones, esters, acids, and other miscellaneous compounds (Table 1). The major compounds predominantly present in the CO were as follows (in order of decreasing content %): linalool (40.24), *trans*-cinnamyl acetate (11.71), camphor (9.38), cinnamaldehyde (6.87), 3-phenyl-2-propenal (4.06), caryophyllene (2.65), coumarin (2.13), bornyl acetate (1.72), limonene (1.53), α-(+)-pinene (1.38), estragole (1.31), and caryophyllene oxide (1.00). These 12 compounds were identified to have content greater than 1%, and combined, they constituted 83.99% of the leaf essential oils analyzed. Four cinnamaldehyde congeners combined constituted 18.96% of oil, including the following (in order of decreasing content %): *trans*-cinnamyl acetate (11.71), cinnamaldehyde (6.87), *cis*-cinnamic acid (0.23), and 2-hydroxybenzaldehyde (0.14) (Table 1). On the basis of our results, the chemotype for the *C. osmophloeum* we tested was the linalool type, which appeared as the major constituent having 40.24% in the essential oil. In contrast, cinnamaldehyde is present only in a relatively small amount compared to that of linalool in the CO we analyzed.

Animal Characteristics. Induced diabetes with streptozotocin was associated with the development of a slower rate of body weight (bw) gain, greater food and water intake, and greater urine excretion with loss of skeletal muscle (Table 2). A significant elevation of liver-weight-to-body-weight ratio was also found in the STZ-induced diabetic rats (Table 2). Compared with that in the vehicle-treated diabetes group, the elevated food intake, water intake, and urine excretion tend to be ameliorated by the CO treatment, in which a low dose was shown to be

Table 2. Metabolic Characteristics and Tissue/Organ Weight/Body Weight Ratio of Control Rats or Streptozotocin-Induced Diabetic Rats That Did or Did Not Receive CO, Cinnamaldehyde, or Glibenclamide[a]

	control-V	DM-V	DM-COL	DM-COM	DM-COH	DM-CA	DM-GBC
bw gain (g)	83.8 ± 20.2	48.1 ± 10.9[c]	65.3 ± 18.1	64.3 ± 16.3	55.7 ± 18.7	51.5 ± 27.4	44.8 ± 37.5
food intake (g/24 h)	22.7 ± 1.3	33.5 ± 3.7[c]b	23.6 ± 0.9a	25.0 ± 2.2ab	25.5 ± 3.7ab	30.5 ± 6.9ab	26.2 ± 12.0ab
water intake (mL/24 h)	52.3 ± 9.6	160.3 ± 23.5[c]b	92.5 ± 16.2a	128.5 ± 25.7ab	123.3 ± 24.2ab	102.5 ± 63.3ab	126.2 ± 20.1ab
urine excretion (mL/24 h)	14.3 ± 1.7	145.8 ± 15.5[c]b	104.2 ± 21.3a	117.4 ± 17.8ab	110.3 ± 22.3ab	100.5 ± 61.2ab	119.3 ± 23.4ab
skeletal muscle w/bw (%)[b]	1.48 ± 0.05	1.18 ± 0.01[c]a	1.46 ± 0.60c	1.34 ± 0.07b	1.40 ± 0.04bc	1.32 ± 0.09b	1.38 ± 0.08bc
liver w/bw (%)	3.52 ± 0.19	4.84 ± 0.26[c]c	3.94 ± 0.26a	3.99 ± 0.44a	4.23 ± 0.24ab	4.53 ± 0.27bc	4.59 ± 0.20bc
spleen w/bw (%)	0.28 ± 0.03	0.31 ± 0.03	0.27 ± 0.03	0.28 ± 0.02	0.27 ± 0.03	0.28 ± 0.03	0.28 ± 0.01

[a]Values are the mean ± SD for six rats per group. Control-V, control rats treated with vehicle; DM-V, DM rats treated with vehicle; DM-COL, DM rats treated with 12.5 mg/(kg bw) of CO; DM-COM, DM rats treated with 25 mg/(kg bw) of CO; DM-COH, DM rats treated with 50 mg/(kg bw) of CO; DM-CA, DM rats treated with 40 mg/(kg bw) of cinnamaldehyde; DM-GBC, DM rats treated with 1.2 mg/(kg bw) of glibenclamide. [b]Sum of gastrocnemius muscle, soleus muscle, and EDL muscle. [c]Significantly different from the control group ($p < 0.05$). a, b, and c indicate the DM groups not sharing the same letter are significantly different ($p < 0.05$).

Table 3. Fasting Blood Glucose Levels of Control Rats or Diabetic Rats That Did or Did Not Receive CO, Cinnamaldehyde, or Glibenclamide on Different Days after Inducing Diabetes[a]

	fasting plasma glucose (mg/dL)						
time after induction	control-V	DM-V	DM-COL	DM-COM	DM-COH	DM-CA	DM-GBC
day 3	88.9 ± 5.2	165.0 ± 36.6[b]	164.9 ± 44.8	165.3 ± 17.5	164.6 ± 46.8	165.7 ± 28.3	165.7 ± 9.7
day 10	89.8 ± 9.8	200.7 ± 41.8[b]a	129.6 ± 34.6b	166.2 ± 52.8ab	160.8 ± 51.4ab	177.0 ± 55.0ab	186.8 ± 73.9a
day 17	89.5 ± 7.1	316.4 ± 88.5[b]a	160.9 ± 32.4b	249.9 ± 76.6a	263.1 ± 119.9a	275.0 ± 95.4a	302.5 ± 81.8a
day 21	89.3 ± 8.7	429.3 ± 61.3[b]a	174.6 ± 47.3b	323.8 ± 88.7c	327.2 ± 61.3c	369.8 ± 109.7ac	393.5 ± 85.5ac

[a]Values are the mean ± SD for six rats per group. Control-V, control rats treated with vehicle; DM-V, DM rats treated with vehicle; DM-COL, DM rats treated with 12.5 mg/(kg bw) of CO; DM-COM, DM rats treated with 25 mg/(kg bw) of CO; DM-COH, DM rats treated with 50 mg/(kg bw) of CO; DM-CA, DM rats treated with 40 mg/(kg bw) of cinnamaldehyde; DM-GBC, DM rats treated with 1.2 mg/(kg bw) of glibenclamide. [b]Significantly different from the control group ($p < 0.05$). a, b, and c indicate the DM groups not sharing the same letter are significantly different ($p < 0.05$).

significantly effective and to a greater extent than that of cinnamaldehyde and glibenclamide.

All tested doses of CO significantly reversed the skeletal muscle loss in diabetic rats in which a low CO dose was shown to be more effective than that of cinnamaldehyde and glibenclamide. Liver enlargement in diabetics was shown to significantly reverse by all tested CO doses; however, such a beneficial effect was not found with either cinnamaldehyde or glibenclamide. Although the CO effect to ameliorate body-weight gain of diabetic rats was nonsignificant compared to that of vehicle-treated rats, the average body-weight gain improved by approximately 36%, 34%, and 15% in rats treated with low, medium, and high CO doses, respectively. The average body-weight gain in diabetic rats only improved for cinnamaldehyde by7%, and the decrease in average body-weight gain is 7% in the glibenclamide-treated group (Table 2).

The STZ injections caused significantly elevated concentrations of fructosamine in plasma compared with that of the control rats and were shown to be 1.17 ± 0.91 and 9.80 ± 4.42 mg/mL for control and diabetes, respectively ($p < 0.05$). The treatment with CO significantly reversed the fructosamine level in diabetic rats and was 2.87 ± 1.00, 2.84 ± 0.47, and 3.60 ± 0.65 mg/dL for low, medium, and high CO doses, respectively. Cinnamaldehyde and glibenclamide also significantly lowered plasma fructosamine levels in diabetics but to a lesser extent than that of CO and were 3.47 ± 1.09 and 3.81 ± 1.51 mg/dL, respectively.

Fasting Plasma Glucose and Plasma and Pancreatic Insulin Levels. During 3−21 d after the STZ injection, the vehicle-treated rats showed time-dependent deteriorated fasting plasma glucose in a time-dependent manner. The CO-treated diabetic rats showed ameliorated fasting blood glucose from day 3 to day 21, compared to that of the vehicle-treated rats, in which a low dose of CO was shown to be significantly effective since day 10, and medium and high doses of CO were shown to be significantly effective at day 21. At day 21, after STZ injection, Cinnamaldehyde and glibenclamide improved the average fasting plasma glucose level by 13.9% and 8.3%, respectively, but were not statistically significant (Table 3).

At the end of the treatment with CO, cinnamaldehyde, or glibenclamide, it showed that all tested doses of CO significantly reversed diabetes-induced lowering of the plasma insulin level in which medium and high doses of CO have a similar effect to that of cinnamaldehyde and glibenclamide, whereas the low CO dose showed a significantly greater effect to elevate plasma insulin than that of high-dose CO, cinnamaldehyde, and glibenclamide (Figure 1A). All tested CO doses significantly elevated the pancreatic insulin level to a similar extent as that of glibenclamide and to a greater extent than that of cinnamaldehyde (Figure 1B).

Oral Glucose Tolerance Test. Blood samples were collected immediately before and during the 120 min period after glucose loading. The integral values of the area under the curve (AUC) of glucose, insulin, GIP, and GLP-1 response during the 120 min period were calculated using the trapezoid method and expressed as the control percentage (Figure 2). Glucose tolerance deteriorated in the vehicle-treated diabetic animals; the AUC of glucose was significantly elevated in the diabetic group compared to that of the control group. However, this deteriorated glucose tolerance was attenuated by treatment with all tested doses of CO in which the low dose was shown to have the greatest beneficial effect among all CO doses and was significantly greater than that of glibenclamide (Figure 2A).

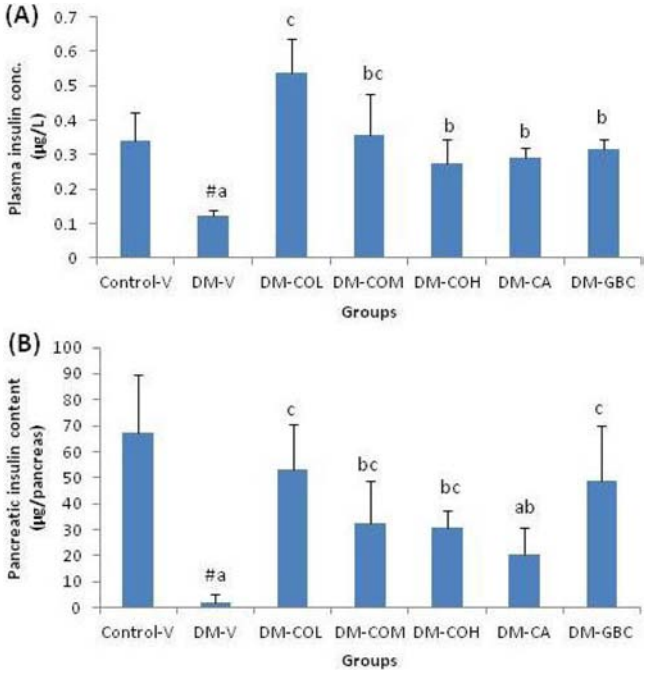

Figure 1. Effect of CO and cinnamaldehyde on insulin levels (A) in peripheral blood and (B) in the pancreas of diabetic rats. Control-V, control rats treated with vehicle; DM-V, DM rats treated with vehicle; DM-COL, DM rats treated with 12.5 mg/(kg bw) of CO; DM-COM, DM rats treated with 25 mg/(kg bw) of CO; DM-COH, DM rats treated with 50 mg/(kg bw) of CO; DM-CA, DM rats treated with 40 mg/(kg bw) of cinnamaldehyde; DM-GBC, DM rats treated with 1.2 mg/(kg bw) of glibenclamide. Data are means ± SDs for six rats in each group. #Significantly different from the control ($p < 0.05$). a, b, c not sharing the same letter are significantly different ($p < 0.05$).

The AUC of plasma insulin during the oral glucose tolerance test for the vehicle-treated diabetic rats was only 13.7% of the control value. However, CO treatment at all tested doses significantly improved the insulin response to oral glucose loading to an extent similar to that of glibenclamide (Figure 2B).

The response of the intestinal hormones GIP and GLP-1 showed that both were significantly lowered in STZ-induced diabetic rats compared with that of the controls. The CO treatment significantly reversed the AUC of GLP-1 to an extent similar to that of glibenclamide (Figure 2C). CO also significantly reversed the AUC of GIP in diabetes rats that was unaffected by glibenclamide (Figure 2D).

Oxidative Stress Parameters in the Pancreas. The lipid peroxidation level reflected by TBARS analysis showed increased oxidative stress in the pancreas of diabetic rats associated with lowered SOD and GRd activity and the total GSH level in this organ ($p < 0.05$). The low-dose CO treatment significantly reversed diabetes-induced elevation of the lipid peroxidation level in the pancreas ($p < 0.05$) (Table 4); however, medium and high doses of CO, cinnamaldehyde, and glibenclamide did not significantly improve the lipid peroxidation level in the pancreas of diabetic rats. All tested CO doses significantly elevated pancreatic SOD and GRd activities, in which low-dose CO was the most effective and improved enzyme activity to an extent similar to that of cinnamaldehyde ($p < 0.05$; Table 4). Glibenclamide significantly elevated GRd activity but not SOD and was less effective than that of low-dose CO and cinnamaldehyde (Table 4). All tested CO doses significantly reversed the lowered total GSH content in the pancreas of diabetic rats to an extent greater than that of cinnamaldehyde, whereas glibenclamide did not show any effect on pancreatic total GSH content (Table 4).

Inflammatory Parameters in the Pancreas. We found that the IL-1β, TNF-α, and NO contents in the pancreas were elevated significantly in diabetics compared with those of the controls ($p < 0.05$). Low and medium doses of CO significantly improved these inflammatory parameters in the pancreas of diabetic rats, in which the low dose was shown to be more effective than the medium dose of CO, and both were shown to provide similar anti-inflammatory effects in lowering pancreatic levels of IL-1β and NO as that of cinnamaldehyde (Table 4). Glibenclamide did not show any effect on these inflammatory parameters in the pancreas of diabetic rats.

DISCUSSION

Numerous recent reports have provided evidence for an antidiabetic effect of cinnamon products, the species of cinnamon focused on *C. cassia*, *C. zeylanicum*, and *C. burmannii*,[6−8,21] among which *C. cassia* is likely the mostly studied. Indigenous cinnamon (*C. osmophloeum* Kanehira) is an economic plant in Taiwan, and it is cultivated in large areas in the country. Researchers have been interested in the cinnamaldehyde type because the chemical constituents of its leaf essential oil are similar to those of *C. cassia* bark oil with cinnamaldehyde as the major chemical composition.[17] However, studies on the antidiabetic action of indigenous cinnamon are rare. A couple of studies have proposed cinnamaldehyde to be a major functional compound for the antidiabetic activity of cinnamon.[15,16] When administered each day for 45 d at 5, 10, or 20 mg/(kg bw), cinnamaldehyde significantly lowered fasting blood glucose in a time-dependent manner, and at 20 mg/(kg bw d), cinnamaldehyde significantly improved body weight, food intake, and glycated hemoglobin level and total cholesterol, and elevated peripheral insulin and HDL-cholesterol in peripheral blood in STZ-DM rats.[15] Thus, we adopted the 40 mg/(kg bw) cinnamaldehyde dose and gavaged to rats every other day to compare with that of CO. Our investigation with cinnamaldehyde showed a similar result as that reported previously of ameliorated blood glucose and fructosamine levels in diabetes.[15] We showed that CO is useful for improving glycemic control in diabetic rats and is associated with ameliorated peripheral and pancreatic insulin content and elevated GIP and GLP-1 levels. Most CO effects were superior to that of glibenclamide, a traditional oral hypoglycemic agent.

According to the GC/MS analysis of our essential oil sample, low-dose CO gavaged to diabetic rats was equivalent to approximately 0.9 mg/(kg bw) of cinnamaldehyde. All parameters investigated with low-dose CO-treated DM rats were ameliorated to a significantly greater extent than that of 40 mg/(kg bw) of cinnamaldehyde, thus suggesting functional compound(s) in addition to cinnamaldehyde present in this essential oil. Linalool was shown to be the major volatile compound in CO, and the CO dose gavaged to diabetic rats was equivalent to approximately 5, 10, or 20 mg/(kg bw) of linalool in low, medium, or high doses of CO, respectively. This is the first report to show a hypoglycemic effect of linalool-type indigenous cinnamon; however, the antidiabetic effect of linalool may not be a novel concept. Ancient Indians used the coriander-seed essential oil that is rich in linalool for treating diabetes, and Deepa and Venkatraman Anuradha[22] recently reported that a treatment with 25 mg/(kg bw) of linalool each day for 45 d significantly improved glycemic control and ameliorated several hyperglycemia-associated complications such as nephropathy progression

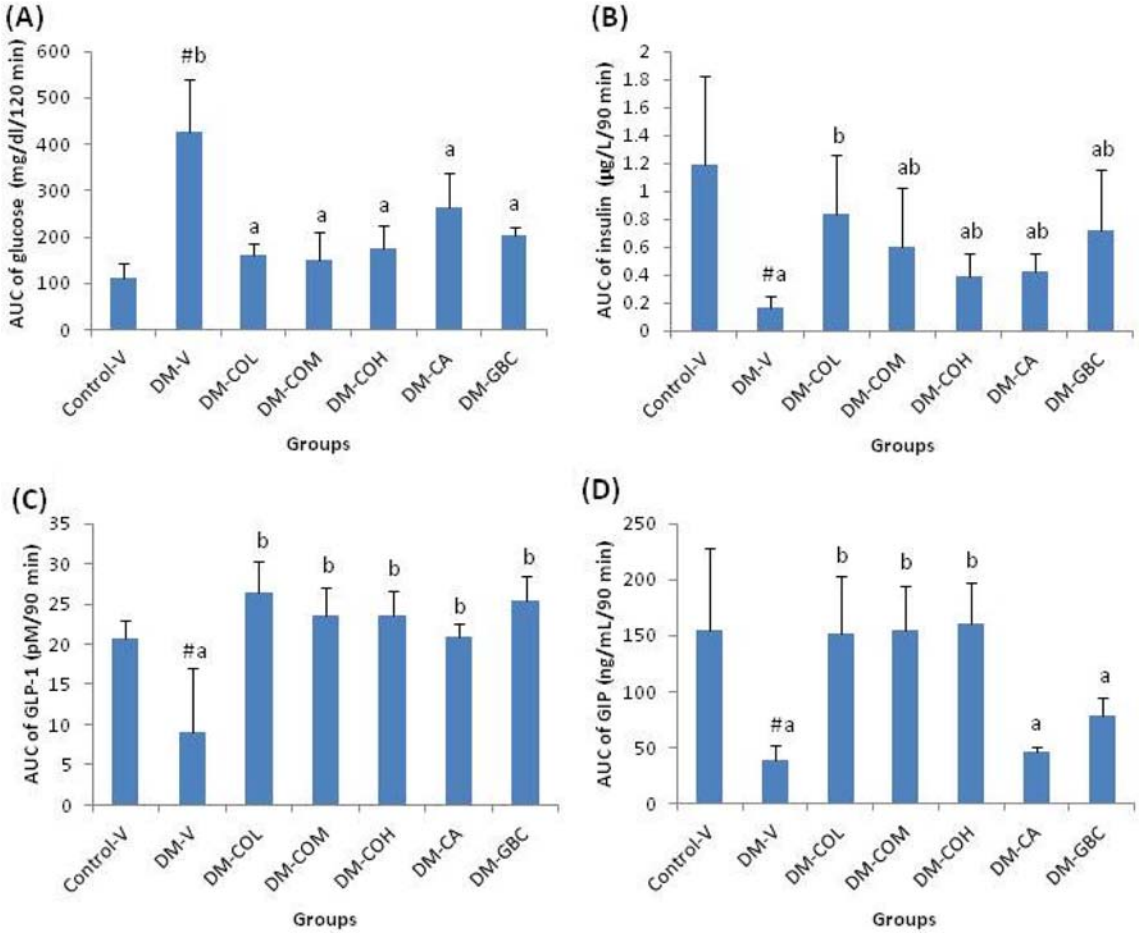

Figure 2. Effect of CO and cinnamaldehyde on the increment in plasma (A) glucose, (B) insulin, (C) GLP-1, and (D) GIP in response to an oral glucose bolus in diabetic rats calculated during the OGTT period. A glucose bolus (1 g/(kg bw)) was administered orally on day 17 after inducing diabetes. Glucose was measured in plasma samples from the tail vein. Control-V, control rats treated with vehicle; DM-V, DM rats treated with vehicle; DM-COL, DM rats treated with 12.5 mg/(kg bw) of CO; DM-COM, DM rats treated with 25 mg/(kg bw) of CO; DM-COH, DM rats treated with 50 mg/(kg bw) of CO; DM-CA, DM rats treated with 40 mg/(kg bw) of cinnamaldehyde; DM-GBC, DM rats treated with 1.2 mg/(kg bw) of glibenclamide. Data are means ± SDs for six rats in each group. #Significantly different from the control ($p < 0.05$). a and b not sharing the same letter are significantly different ($p < 0.05$).

in STZ-DM rats. Compared with cinnamaldehyde, low-dose CO showed a greater beneficial effect on glycemic control and pancreatic function. According to Reagan-Shaw et al.,[23] because of the different metabolic rate among different animal species, the dose translation from rat to human adult are suggested to be corrected by a factor 6/37; therefore, the low dose of CO used in the present study with rats would correspond to about 4 mg/kg for a human adult. Since the hypoglycemic effect of CO is reflected by higher pancreatic and peripheral insulin content at fasting and during the OGTT period, we consequently propose that CO helps protect the pancreas from hyperglycemic-associated damage caused by elevated oxidative stress and a proinflammatory environment in diabetes.

Oxidative stress appears to be critically involved in impaired β-cell function during diabetes development.[24] Similar to previous study findings, we found that, in STZ-DM rats, pancreatic antioxidant defenses were significantly lowered. We showed that, in a diabetic condition, the treatment with an appropriate CO dose is effective to ameliorate oxidative stress parameters in the pancreas to an extent greater than that of glibenclamide. This result partially explains the finding that an appropriate CO dose showed a greater effect on glycemic control than that of glibenclamide.

Low-grade inflammation in type 2 diabetes mellitus might contribute to β-cell failure and diabetes development.[25] Studies with human islets isolated from pancreases have shown that in vitro treatment of β cells with an IL-1 receptor antagonist improves β-cell survival and secretory function.[26] Inducible nitric oxide synthase (iNOS), a major mediator of inflammation whose synthesis is markedly increased by elevating iNOS expression induced by proinflammatory cytokines and ROS, has been implicated in β-cell damage in diabetes.[27] Chao et al.[28] and Tung et al.[29] used LPS-treated J774A.1 andRAW264.7 murine macrophages to show the anti-inflammatory activity of the indigenous cinnamon. We showed that CO partially reverses elevated IL-1β, TNF-α, and NO content in the pancreas of STZ-DM rats. Thus, the effect of CO to ameliorate β-cell function in diabetic rats can also partially be explained by its anti-inflammatory action.

We showed that linalool is the major composition of CO. Linalool was reported to ameliorate H_2O_2-induced lipid peroxidation in porcine brain[30] and to reverse the elevated level of lipid peroxidation and the lowered activities of glutathione peroxidase and SOD in STZ-injected rats.[22] Studies investigating the anti-inflammatory activity of linalool are scant. Peana et al.[31] reported that linalool inhibited LPS-induced J774.A1 macrophages to produce NO, prostaglandin E2, and

Table 4. Parameters of Oxidative Stress and Inflammatory Condition in the Pancreas of Control Rats or Streptozotocin-Induced Diabetic Rats That Did or Did Not Receive CO, Cinnamaldehyde, or Glibenclamide[a]

	control-V	DM-V	DM-COL	DM-COM	DM-COH	DM-CA	DM-GBC
TBARS (nmol/mg protein)	0.16 ± 0.04	0.34 ± 0.08[b]a	0.19 ± 0.04 b	0.41 ± 0.09 a	0.37 ± 0.11 a	0.37 ± 0.1 a	0.33 ± 0.04 a
SOD (U/mg protein)	0.38 ± 0.05	0.07 ± 0.02[b]a	0.24 ± 0.03 b	0.16 ± 0.03 c	0.13 ± 0.02 c	0.22 ± 0.04 b	0.08 ± 0.01 a
GPx (nmol NADPH/(min mg protein))	42.1 ± 9.3	39.5 ± 11.0 a	44.7 ± 7.5 ab	45.0 ± 7.4 ab	49.6 ± 9.8 b	48.8 ± 6.8 b	44.5 ± 5.9 ab
GRd (nmol NADPH/(min mg protein))	22.5 ± 1.8	11.5 ± 1.7[b]a	19.0 ± 0.7 b	14.9 ± 1.2 c	15.9 ± 1.6 c	17.7 ± 1.9 b	15.5 ± 0.8 c
total GSH (nmol/mg protein)	0.092 ± 0.015	0.005 ± 0.001[b]a	0.040 ± 0.008 b	0.038 ± 0.006 b	0.034 ± 0.007 b	0.018 ± 0.006 c	0.006 ± 0.002 a
IL-1β (pg/mg protein)	4905 ± 1743	13287 ± 4169[b]a	5284 ± 994 b	9486 ± 3201 c	12638 ± 2026 a	7915 ± 1734 bc	13008 ± 2800 a
TNF-α (pg/mg protein)	2048 ± 575	4258 ± 1266[b]a	2358 ± 875 a	3326 ± 1222 ab	3667 ± 833 a	3924 ± 1330 a	4272 ± 1267 a
NO (nmol/mg protein)	6.70 ± 0.49	8.27 ± 1.14[b]a	4.66 ± 0.90 b	5.37 ± 0.98 b	7.57 ± 1.61 a	5.12 ± 1.05 b	7.84 ± 1.85 a

[a]Values are the mean ± SD for six rats per group. Control-V, control rats treated with vehicle; DM-V, DM rats treated with vehicle; DM-COL, DM rats treated with 12.5 mg/kg bw of CO; DM-COM, DM rats treated with 25 mg/kg bw of CO; DM-COH, DM rats treated with 50 mg/kg bw of CO; DM-CA, DM rats treated with 40 mg/kg bw of cinnamaldehyde; DM-GBC, DM rats treated with 1.2 mg/kg bw of glibenclamide. [b]Significantly different from the control group ($p < 0.05$). a, b, and c indicate the DM groups not sharing the same letter are significantly different ($p < 0.05$).

cyclooxygenase-2. Deepa and Venkatraman Anuradha[22] found that linalool reversed hepatic pro-inflammatory cytokine levels in STZ-DM rats. As to the other functional compound of CO, cinnamaldehyde, we found that it improved pancreatic SOD and GRd activity that is consistent with the findings by others.[32] However, we found that the SOD contribution may be too small in the antioxidant system of the pancreas and cinnamaldehyde did not significantly improve the total GSH, which may explain why cinnamaldehyde showed a less protective effect on pancreatic lipid peroxidation. Cinnamaldehyde has also been reported to suppress LPS-induced macrophage and monocyte IL-1β, TNF-α, and NO production.[28,33] Consistent with these findings, we confirmed cinnamaldehyde anti-inflammatory activity in the pancreas of the STZ-DM rat model. Thus, we interpreted that linalool and cinnamaldehyde both contribute to the anti-inflammatory activity of CO but that the role of other constituents cannot be excluded.

Although we found that CO at low and medium doses provided significant benefit in controlling diabetes, high-dose CO did not show better activity than that of lower-dose CO for improving plasma insulin levels at fasting and during the OGTT period. This result suggests that certain CO components may disturb the beneficial effect of high-dose CO, and we thus proposed camphor as a candidate. According to our GC/MS analysis, camphor represents 9.38% of the composition in CO, and the rats gavaged with high-dose CO received camphor at a dose equivalent to approximately 5 mg/(kg bw). Camphor is a common natural product, historically used as a remedy for several symptoms. A pharmacological study showed that camphor suppresses oxidative metabolism, and when given to mice at 300 mg/kg for 20 d, it elevates hepatic phase I and phase II enzyme activity, reduces the GSH level, and inhibits mitochondrial respiration.[34] The pancreatic β-cell insulin secretion response to glucose stimulation largely relies on glucose oxidation in these cells.[35] Whether camphor at high-dose CO might produce less insulin secretion through the inhibition of mitochondrial respiration and worsen the subsequent hyperglycemic condition compared to that of lower CO doses warrants further investigation. In conclusion, our study is the first to report that an appropriate dose of leaf essential oil of the linalool type indigenous cinnamon possesses a hypoglycemic effect in diabetes and is more effective than that of cinnamaldehyde and glibenclamide. For an adult of 60 kg body weight, the effective dose of CO at 240 mg would be recommended. The hypoglycemic effect of CO is at least partly via increased insulin secretion. CO ameliorated oxidative stress and the pro-inflammatory environment in pancreas suggesting a possible protective effect on pancreatic β cells. High-dose CO may provide an effect that offsets its beneficial effect in diabetes and warrants further investigation.

AUTHOR INFORMATION

Corresponding Author

*Fax: +886 423248175; e-mail: ctl@csmu.edu.tw.

Author Contributions

⊥S.-C.L. and W.-X.X. contributed equally to this work.

Funding

This work was supported by a grant from the NSC foundation of the National Science Council (NSC98-2320-B-040-017-MY3).

Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

AUC, area under curve; CO, leaf essential oil from indigenous cinnamon; DM, diabetes mellitus; GC/MS, gas chromatography−mass spectrometry; GIP, gastric inhibitory peptide; GLP-1, glucagon-like peptide-1; GRd, glutathione reductase; GSH, glutathione; IL, interleukin; LPS, lipopolysaccharide; NO, nitric oxide; OGTT, oral glucose tolerance test; ROS, reactive oxygen species; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances; TNF, tumor necrosis factor; STZ, streptozotocin

REFERENCES

(1) Ceriello, A.; Bortolotti, N.; Falleti, E. Total radical-trapping antioxidant parameter in non-insulin-dependent diabetic patients. *Diabetes Care* **1997**, *20*, 194−197.

(2) Bonnefont-Rousselot, D.; Bastard, J. P.; Jaudon, M. C.; Delattre, J. Consequences of the diabetic status on the oxidant/antioxidant balance. *Diabetes Metab.* **2000**, *26*, 163−176.

(3) Ramakrishna, V.; Jaikhani, R. Oxidative stress in non-insulin-dependent diabetes mellitus (NIDDM) patients. *Acta Diabetol.* **2008**, *45*, 41−46.

(4) Hotamisligil, G. S. Inflammation and metabolic disorders. *Nature* **2006**, *444*, 860−867.

(5) Esposito, K.; Nappo, F.; Marfella, R.; Giugliano, G.; Giugliano, F.; Ciotola, M.; Quagliaro, L.; Ceriello, A.; Giugliano, D. Inflammatory cytokine concentrations are acutely increased by hyperglycemia in humans: Role of oxidative stress. *Circulation* **2002**, *106*, 2067−2072.

(6) Jakhetia, V.; Pate, R.; Khatri, P.; Pahuj, N.; Garg, S.; Pandey, A.; Sharma, S. Cinnamon: A pharmacological review. *J. Adv. Sci. Res.* **2010**, *1*, 19−23.

(7) Ranasinghe, P.; Jayawardana, R.; Galappaththy, P.; Constantine, G. R.; de Vas Gunawardana, N.; Katulanda, P. Efficacy and safety of 'true'

cinnamon (*Cinnamomum zeylanicum*) as a pharmaceutical agent in diabetes: A systematic review and meta-analysis. *Diabetic Med.* **2012**, *29*, 1480−1492.
(8) Akilen, R.; Tsiami, A.; Devendra, D.; Robinson, N. Cinnamon in glycaemic control: Systematic review and meta analysis. *Clin. Nutr.* **2012**, *31*, 609−615.
(9) Davis, P. A.; Yokoyama, W. Cinnamon intake lowers fasting blood glucose: Meta-analysis. *J. Med. Food* **2011**, *14*, 884−889.
(10) Vanschoonbeek, K.; Thomassen, B. J. W.; Senden, J. M.; Wodzig, W. K. W. H.; van Loon, L. J. C. Cinnamon supplementation does not improve glycemic control in postmenopausal type 2 diabetes patients. *J. Nutr.* **2006**, *136*, 977−980.
(11) Blevins, S. M.; Leyva, M. J.; Brown, J.; Wright, J.; Scofield, R. H.; Aston, C. E. Effect of cinnamon on glucose and lipid levels in non-insulin dependent type 2 diabetes mellitus. *Diabetes Care* **2007**, *30*, 2236−2237.
(12) Baker, W. L.; Gutierrez-Williams, G.; White, C. M.; Kluger, J.; Coleman, C. I. Effect of cinnamon on glucose control and lipid parameters. *Diabetes Care* **2008**, *31*, 41−43.
(13) Ziegenfuss, T. N.; Hofheins, J. E.; Mendel, R. W.; Landis, J.; Anderson, R. A. Effects of a water-soluble cinnamon extract on body composition and features of the metabolic syndrome in pre-diabetic men and women. *J. Int. Soc. Sports Nutr.* **2006**, *3*, 45−53.
(14) Kim, W.; Khil, L. Y.; Clark, R.; Bok, S. H.; Kim, E. E.; Lee, S.; Jun, H. S.; Yoon, J. W. Naphthalenemethyl ester derivative of dihydroxyhydrocinnamic acid, a component of cinnamon, increases glucose disposal by enhancing translocation of glucose transporter 4. *Diabetologia* **2006**, *49*, 2437−2448.
(15) Subash Babu, P.; Prabuseenivasan, S.; Ignacimuthu, S. Cinnamaldehyde—A potential antidiabetic agent. *Phytomedicine* **2007**, *14*, 15−22.
(16) Sangal, A. Role of cinnamon as beneficial antidiabetic food adjunct: A review. *Adv. Appl. Sci. Res.* **2011**, *2*, 440−450.
(17) Cheng, S. S.; Liu, J. Y.; Hsui, Y. R.; Chang, S. T. Chemical polymorphism and antifungal activity of essential oils from leaves of different provenances of indigenous cinnamon (*Cinnamomum osmophloeum*). *Bioresour. Technol.* **2006**, *97*, 306−312.
(18) Schonburg, G.; Dielmann, G. Identification by means of retention parameters. *J. Chromatogr. Sci.* **1973**, *11*, 51−159.
(19) Liu, C. T.; Hse, H.; Lii, C. K.; Chen, P. S.; Sheen, L. Y. Effects of garlic oil and diallyl trisulfide on glycemic control in rats with streptozotocin-induced diabetes. *Eur. J. Pharmacol.* **2005**, *516*, 165−173.
(20) Liu, C. T.; Hsu, T. W.; Chen, K. M.; Tan, Y. P.; Lii, C. K.; Sheen, L. Y. The antidiabetic effect of garlic oil is associated with ameliorated oxidative stress but not ameliorated level of pro-inflammatory cytokines in skeletal muscle of streptozotocin-induced diabetic rats. *J. Tradit. Complementary Med.* **2012**, *2*, 135−144.
(21) Leach, M. J.; Kumar, S. Cinnamon for diabetes mellitus. *Cochrane Database Syst. Rev.* **2012**, *9*, CD007170.
(22) Deepa, B.; Venkatraman Anuradha, C. Linalool, a plant derived monoterpene alcohol, rescues kidney from diabetes-induced nephropathic changes via blood glucose reduction. *Diabetol. Croat.* **2011**, *40*−*4*, 121−137.
(23) Reagan-Shaw, S.; Nihal, M.; Ahmad, N. Dose translation from animal to human studies revisited. *FASEB J.* **2007**, *22*, 659−661.
(24) Drews, G.; Krippeit-Drews, P.; Düfer, M. Oxidative stress and beta-cell dysfunction. *Pflügers Arch. − Eur. J. Physiol.* **2010**, *460*, 703−718.
(25) Donath, M. Y.; Schumann, D. M.; Faulenbach, M.; Ellingsgaard, H.; Perren, A.; Ehses, J. A. Islet inflammation in type 2 diabetes. *Diabetes Care* **2008**, *31*, S161−S164.
(26) Ardestani, A.; Sauter, N. S.; Paroni, F.; Dharmadhikari, G.; Cho, J. H.; Lupi, R.; Marchetti, P.; Oberholzer, J.; Conte, J. K.; Maedler, K. Neutralizing interleukin-1beta (IL-1beta) induces beta-cell survival by maintaining PDX1 protein nuclear localization. *J. Biol. Chem.* **2011**, *286*, 17144−17155.
(27) Hughes, K. J.; Chambers, K. T.; Meares, G. P.; Corbett, J. A. Nitric oxides mediates a shift from early necrosis to late apoptosis in cytokine-treated β-cells that is associated with irreversible DNA damage. *Am. J. Physiol.* **2009**, *297*, E1187−E1196.
(28) Chao, L. K.; Hua, K. F.; Hsu, H. Y.; Cheng, S. S.; Liu, J. Y.; Chang, S. T. Study on the antiinflammatory activity of essential oil from leaves of *Cinnamomum osmophloeum*. *J. Agric. Food Chem.* **2005**, *53*, 7274−7278.
(29) Tung, Y. T.; Chua, M. T.; Wang, S. Y.; Chang, S. T. Anti-inflammation activities of essential oil and its constituents from indigenous cinnamon (*Cinnamomum osmophloeum*) twigs. *Bioresour. Technol.* **2008**, *99*, 3908−3913.
(30) Celik, S.; Ozkaya, A. Effects of intraperitoneally administered lipoic acid, vitamin E, and linalool on the level of total lipid and fatty acids in guinea pig brain with oxidative stress induced by H_2O_2. *J. Biochem. Mol. Biol.* **2002**, *35*, 547−552.
(31) Peana, A. T.; Marzocco, S.; Popolo, A.; Pinto, A. (−)-Linalool inhibits in vitro NO formation: Probable involvement in the antinociceptive activity of this monoterpene compound. *Life Sci.* **2006**, *78*, 719−723.
(32) Liao, J. C.; Deng, J. S.; Chiu, C. S.; Hou, W. C.; Huang, S. S.; Shie, P. H.; Huang, J. Anti-inflammatory activities of *Cinnamomum cassia* constituents in vitro and in vivo. *J. Evidence-Based Complementary Altern. Med.* **2012**, 429320.
(33) Chao, L. K.; Hua, K. F.; Hsu, H. Y.; Cheng, S. S.; Lin, I. F.; Chen, C. J.; Chen, S. T.; Chang, S. T. Cinnamaldehyde inhibits pro-inflammatory cytokines secretion from monocytes/macrophages through suppression of intracellular signaling. *Food Chem. Toxicol.* **2008**, *46*, 220−231.
(34) Zuccarini, P. Camphor: Risks and benefits of a widely used natural product. *J. Appl. Sci. Environ. Manage.* **2009**, *13*, 69−74.
(35) Wollheim, C. B. Beta-cell mitochondria in the regulation of insulin secretion: A new culprit in Type II diabetes. *Diabetologia* **2000**, *43*, 265−277.