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Antiatherogenic activity of *Dendropanax morbifera* essential oil in rats

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In Korea, *Dendropanax morbifera* Leveille (Araliaceae) is commonly used in traditional medicines for various diseases. We evaluated the hypolipidemic activity of *D. morbifera* essential oil (DMEO) in male Wistar rats (weight, 160 ± 15 g) maintained on a high-cholesterol diet. DMEO was extracted by hydrodistillation and analyzed using gas chromatography/mass spectroscopy (GC/MS). The DMEO yield was 3.5%, and GC/MS analysis revealed that its major constituents were γ-elemene (18.59%), tetramethyltricyclo hydrocarbons (10.82%), β-zingiberene (10.52%), and β-selinene (10.41%). Rats were orally administered DMEO at doses of 50, 100, and 200 $mg \cdot kg^{-1} \cdot d^{-1}$ for 2 weeks. DMEO significantly and dose-dependently reduced the total cholesterol, triglyceride, and low-density lipoprotein cholesterol levels and significantly increased the high-density lipoprotein cholesterol levels. We conclude that *D. morbifera* has significant lipid-lowering effects and is a promising agent that should be considered in studies seeking new, safe, and effective natural cardioprotective agents.

1. Introduction

Heart disease has been and remains the leading cause of death in most countries. Numerous population studies have reported that elevated plasma total cholesterol (TC) or low-density lipoprotein cholesterol (LDL-C) levels are associated with an increased incidence of atherosclerotic events. Epidemiological studies have established a direct relationship between the serum cholesterol levels and coronary artery disease (Key., 1975). Several experimental and interventional studies have confirmed that lowering the serum cholesterol and lipid levels to satisfactory levels reduces the morbidity and mortality associated with coronary heart disease (Jackson and Beagle., 1995). Cholesterol-lowering drugs function by reducing elevated LDL-C and TG levels and increasing reduced high-density lipoprotein cholesterol (HDL-C) levels. Simple and sensitive *in vitro* assay systems are essential for efficient screening of active agents. Although many such systems have been developed for screening hypolipidemic agents, the results obtained do not necessarily match those of *in vivo* experiments. Animals maintained on high-fat diets exhibit considerably elevated serum LDL-C, TG, and TC levels and slightly altered HDL-C levels (Morishita et al., 1986). Such animal models have been used to test the effects of various hypolipidemic agents on serum LDL-C, HDL-C, TG, and TC levels (Han et al., 2005). High-fat diets also induce liver damage in animal models (el-Saadany et al., 1991).

Dendropanax morbifera Leveille (Araliaceae) is endemic to the south-western part of South Korea (Han et al., 1998). The roots, leaves, seeds, and stems of this plant are used in folk medicines for headache, infectious diseases and general debility (Bae, 2000). Polyacetylene compounds isolated from *D. morbifera* stems are reported to exhibit anticomplement activity (Park et al., 2004). However, the biological activity of *D. morbifera* has not been investigated in detail. This study aimed to investigate the hypolipidemic activity of *D. morbifera* essential oil (DMEO) in rats.

2. Investigations, results and discussion

The *D. morbifera* flowers yielded 0.72% (v/w) essential oil with a foul odor. Table 1 lists its major chemical constituents, as identified by GC and GC/MS analyses. In their order of elution from the column, these compounds were γ-elemene (18.59%), tetramethyltricyclo hydrocarbons (10.82%), β-selinene (10.41%), and β-zingiberene (10.52%). DMEO

dose-dependently reduced the serum TC, LDL-C, and TG levels of the test rats. The Lipid Research Clinics Primary Prevention Trial has indicated that the plasma LDL-C concentrations and the risk of coronary artery disease are positively correlated (Lipid Research Clinics Program, 1984). The fact that DMEO significantly lowered the serum TC, LDL-C, and TG levels of our test animals indicates that it is a promising protective agent against coronary artery disease. Drugs such as statins lower the serum TC and LDL-C levels by inhibiting the activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase, a rate-limiting enzyme in cholesterol biosynthesis (Alberts et al., 1980). However, the mechanism underlying DMEO's hypocholesterolemic effects is unclear, and further experiments are underway to elucidate this. Several studies reveal that elevated serum HDL-C levels are associated with a decreased risk of coronary disease (Harrison et al., 2003). Besides lowering the TC, LDL-C, and TG levels, DMEO significantly increased the HDL-C levels and may therefore be an effective agent for the prevention of atherosclerosis and the management of coronary artery disease. Additional experiments are required to clarify the mechanism underlying DMEO's hypolipidemic activity.

3. Experimental

3.1. Plant material and essential oil extraction

Fresh *D. morbifera* flowers were provided by Korea Horticulture Sapling Company (Jeon-Ju, Jeollabuk-Do, South Korea) in July 2007. A voucher specimen has been deposited into the herbarium of this company. The flowers were hydrodistilled for 6 h on a Clevenger-type apparatus. The essential oil obtained was dried over anhydrous sodium sulfate, and this purified oil was stored in an amber-colored vial at 4 °C until further analysis and for use in the serum lipid levels assay.

3.2. Gas chromatography/mass spectroscopy analysis of the essential oil

Gas chromatography/mass spectroscopy (GC/MS) analysis of the essential oil was performed using a QP2010 spectrometer equipped with a splitless injector. The analytes were separated on a DB-1 MS capillary column (0.32 mm (internal diameter) × 60 m; Agilent Scientific) with a film thickness of 0.25 μm. The injector temperature was 300 °C. The initial column temperature was 80 °C for 5 min and was then increased to 280 °C at a rate of 5.0 °C/min and maintained at this temperature for 10 min. Helium was used as the carrier gas (flow rate, 1.0 mL/min). The sample (1 mL diluted 1 : 10 with acetone) was injected in the split mode at a ratio of 1 : 100. The percentage composition of DMEO was calculated from the GC peak areas. The ion source temperature was set at 200 °C and injector temperature, 210 °C. The interface temperature was maintained at 280 °C, and mass spectra were obtained at 70 eV. The column effluent was directly introduced into the ion source. The sector mass analyzer was adjusted to scan 50–500 amu every 0.5 s. The DMEO components were identified by comparing each mass spectral peak with those of authentic samples in a mass spectral library (Wiley Registry of Mass Spectral Data, 7th ed.).

Table 1: Major constituents of DMEO

Retention time*	Components	M^+	Peak area (%)	Fragments	
2.01	γ-Elemene	204	18.59	93	133
2.12	Tetramethyltricyclo hydrocarbons	204	10.82	161	189
2.14	β-Selinene	204	10.41	93	121
2.24	β-Zingiberene	204	10.52	161	119

*Retention time relative to that of α-pinene

3.3. Animals

Male Wistar rats (weight, 160 ± 15 g) were purchased from Orient Bio Inc. (Sungnam, South Korea) and maintained under standard environmental conditions, with free access to water. All experiments conducted in this study were approved by the Animal Care Committee of the Wonkwang University School of Medicine. They were grouped as described by Hajhashemi and Abbasi (2008). Two groups (control and DMEO treated; n = 8) were used to study the effect of DMEO on the serum lipid concentrations of normolipidemic rats. Both groups were maintained on a standard diet for 2 weeks. Rats in the DMEO-treated group were orally administered the agent ($100\ mg \cdot kg^{-1} \cdot d^{-1}$ for 2 weeks). We also assessed the effect of DMEO on the serum lipid concentrations in 5 groups of rats (n = 8) maintained on a high-cholesterol diet (normal diet supplemented with 2% cholesterol and 0.5% cholic acid) for 2 weeks. Group I was the control group. Groups II, III, and IV orally received DMEO at doses of 50, 100, and $200\ mg \cdot kg^{-1} \cdot d^{-1}$, respectively. Group V orally received clofibrate ($100\ mg \cdot kg^{-1} \cdot d^{-1}$). The vehicle (isotonic saline), DMEO, or clofibrate was administered for 2 weeks. After this period, the animals were killed, and serum samples were obtained and maintained frozen at −20 °C until further analysis.

3.4. Determination of serum lipid levels

The serum TC, TG, and HDL-C levels were colorimetrically measured (Bucolo and David, 1973; Allain et al., 1974; Rifai et al., 1992), using commercially available kits (Asan Pharmaceutical, South Korea). The LDL-C levels were estimated using Friedewald's (1972) formula: LDL-C = TC − [HDL-C + (TG/5)]. The atherogenic index was calculated as (TC − HDL-C)/HDL-C (Choi et al., 1991).

3.5. Statistical analysis

The data were statistically analyzed by a one-way analysis of variance, followed by Duncan's test. The results are presented as the mean ± standard deviation (SD).

Table 2: Effect of DMEO on the serum lipid concentrations of rats maintained on a normal diet

Groups	TC (mg/dL)	LDL-C (mg/dL)	HDL-C (mg/dL)	TG (mg/dL)
Control	80.1 ± 2.3	22.2 ± 2.0	42.3 ± 5.3	77.2 ± 5.3
DMEO treated ($100\ mg \cdot kg^{-1} \cdot d^{-1}$)	77.2 ± 1.9	15.8 ± 3.7	47.3 ± 7.3	70.2 ± 3.8

The values are the mean ± SD.

Table 3: Effect of DMEO on the serum lipid profiles of hyperlipidemic rats

Groups	Dose ($mg \cdot kg^{-1} \cdot d^{-1}$)	TC (mg/dL)	LDL-C (mg/dL)	HDL-C (mg/dL)	TG (mg/dL)	AI
Control	0	110.2 ± 5.8	48.2 ± 4.2	40.1 ± 7.6	109.3 ± 9.2	1.74
DMEO treated	50	91.2 ± 3.5[b]	29.1 ± 8.3[a]	46.2 ± 5.3[a]	79.3 ± 8.7[b]	0.97
	100	89.1 ± 4.7[b]	27.4 ± 5.3[b]	47.2 ± 3.2[a]	72.5 ± 5.8[b]	0.88
	200	81.3 ± 5.8[b]	14.5 ± 8.3[b]	53.1 ± 7.8[a]	68.3 ± 7.7[b]	0.53
Clofibrate treated	100	109.3 ± 12.3	47.2 ± 8.3	44.2 ± 6.8	89.1 ± 9.3[a]	1.47

The values are the mean ± SD. AI, atherogenic index.
[a]$p < 0.01$; [b]$p < 0.001$ compared to the control group

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References

Alberts AW, Chen J, Kuron G, Hunt V, Huff J, Hoffman C, Rothrock J, Lopez M, Joshua H, Harris E, Patchett A, Monaghan R, Currie S, Stapley E, Albers-Schonberg G, Hensens O, Hirshfield J, Hoogsteen K, Liesch J, Springer J. (1980) Mevinolin: a highlypotent competitive inhibitor of hydroxymethylglutarylcoenzymeA reductase and a cholesterol-lowering agent. Proc Natl Acad Sci USA 77: 3957–3961.

Allain CC, Poon LS, Chan GS, Richmond W, Fu PC. (1974) Enzymatic determination of total serum cholesterol. Clin Chem 20: 470–475.

Bae KH. (2000) The Medicinal Plants of Korea. Kyo-Hak Pubilishing Co, Ltd, Seoul, Korea, p. 364.

Bucolo G, David H. (1973) Quantitative determination of serum triglycerides by the use of enzymes. Clin Chem 19: 476–482.

Choi JS, Yokozawa T, Oura H. (1991) Antihyperlipidemic effect of flavonoids from Prunus davidiana. J Nat Prod 54: 218–224.

el-Saadany SS, el-Massry RA, Labib SM, Sitohy MZ. (1991) The biochemical role and hypocholesterolaemic potential of the legume Cassia fistula in hypercholesterolaemic rats. Nahrung 35: 807–815.

Friedwald WT, Levy IR, Friedrickson SD. (1972) Estimation of concentration of low density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 18: 499–502.

Hajhashemi V, Abbasi N. (2008) Hypolipidemic Activity of *Anethum graveolens* in Rats *Phytother. Res.* 22: 372–375.

Han LK, Zheng YN, Yoshikawa M, Okuda H, Kimura Y. (2005) Anti-obesity effects of chikusetsusaponins isolated from Panax japonicus rhizomes. BMC Complement Altern Med 5: 9.

Han SH, Jung TH, Oh MH, Ko MH, Oh YS, Koh SC, Kim MH, Oh MY. (1998) Phytogenetic relationships of the *Dendropanax morbifera* and *D. trifidus* based on PCR-RAPD. Kor. J Genetics 20: 173–181.

Harrison D, Kathy KG, Hornig B, Drexler H. (2003) Role of oxidative stress in atherosclerosis. Am J Cardiol 91: 7A–11A.

Jackson R, Beagle HR. (1995) EVIDENCE-BASED MANAGEMENT OF DSYLIPIDAEMIA. Lancet. 346: 1440–1444.

Keys A. (1975) Coronary heart disease, the global picture. Atherosclerosis 22: 149–192.

Lipid Research Clinics Program. (1984) The lipid research clinics coronary primary prevention trial results. i. Reduction in incidence of coronary heart disease. JAMA 251: 251–364.

Morishita S, Saito T, Mishima Y, Mizutani A, Hirai Y, Koyama S, Kawakami M. (1986) Strains and species differences in experimental hyperlipidemia. Nippon Yakurigaku Zasshi. 87: 259–264.

Park BY, Min BS, Oh SR, Kim JH, Kim TJ, Kim DH, Bae KH, Lee HK. (2004) Isolation and anticomplement activity of compounds from *Dendropanax morbifera.* J Ethnopharmacol. 90: 403–408.

Rifai N, Warnick GR, McNamara JR, Belcher JD, Grinstead GF, Frantz IDJR. (1992) Measurement of low density lipoprotein cholesterol (LDL-C) in serum. Clin Chem 38: 150–160.