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Antihypertensive and Cardiovascular Effects of Catechin-Rich Oil Palm (*Elaeis guineensis*) Leaf Extract in Nitric Oxide–Deficient Rats

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ABSTRACT Oil palm (*Elaeis guineensis*) leaf extract (OPLE) possesses good *ex vivo* vasodilation and antioxidant properties. This study evaluated the catechin-rich OPLE antioxidant, antihypertensive, and cardiovascular effects in normal and nitric oxide (NO)–deficient hypertensive rats. OPLE was administered orally (500 mg/kg of body weight/day) to normotensive Wistar rats and N^{ω}-nitro-L-arginine methyl ester (L-NAME)-induced NO-deficient hypertensive rats. OPLE significantly ($P < .05$) attenuated blood pressure increases, increased serum NO, reduced lipid peroxidation, and showed antioxidant effects in NO-deficient hypertensive rats. OPLE decreased the coronary arteriole wall-to-lumen ratio to near normal values under NO deficiency. Although OPLE showed good antihypertensive and antioxidant effects under NO deficiency, it was not hypotensive to normal rats and produced no chronic cardiovascular toxicity in any of the rats throughout the 12-week study. This is the first report on the *in vivo* antihypertensive properties of green tea catechins extracted from an alternative source, namely, oil palm leaf, for use as a medicinal food for hypertension and cardiovascular ailments.

KEY WORDS: • ***antihypertensive*** • ***cardiovascular protective*** • ***catechin*** • **Elaeis guineensis** • ***nitric oxide deficiency***

INTRODUCTION

Methanolic oil palm (*Elaeis guineensis*) leaf extract (OPLE) is rich in polyphenols.[1] The health benefits of green tea catechins for the prevention of cancer,[2,3] inflammation,[4] arthritis,[5] bacterial infections,[6] angiogenesis,[7] oxidation,[8] viral infections,[9] neurodegeneration,[10] and hypercholesterolemia[11] have been well documented.[12] However, the scarce reports on the antihypertensive effects of catechins *in vivo* warrant further investigation and confirmation. The polyphenol-rich OPLE showed vasodilative properties on noradrenaline-preconstricted rat aorta and mesenteric arterial bed, mainly via endothelium-dependent mechanisms.[13] The OPLE also effectively inhibited low-density lipoprotein oxidation better than other edible plant extracts.[14]

The demand for cost-effective, safe, and effective medicinal foods for treating hypertension and cardiovascular disease is increasing. Palm leaves are underutilized by-products of the palm oil industry that can be a potential source of beneficial compounds. The discovery of the *ex vivo* vasodilation and antioxidant properties of OPLE initiated this investigation on the *in vivo* antihypertensive and antioxidative effects of OPLE in nitric oxide (NO)–deficient hypertensive rats. However, sometimes *ex vivo* effects are not reproduced in whole animals. The total OPLE was used because pure compounds may not exert similar vasorelaxant and antioxidative effect like the whole extract.

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MATERIALS AND METHODS

Materials

All chemicals were of analytical grade and were from Sigma-Aldrich (Kuala Lumpur, Malaysia). Captopril, N^{ω}-nitro-L-arginine methyl ester (L-NAME), thiobarbituric acid, and 1,1,3,3-tetraethoxypropane were from Sigma-Aldrich. Ketamine and xylazine were from the Veterinary Hospital, Faculty of Veterinary Medicine, Universiti Putra Malaysia, Serdang, Selangor, Malaysia. Pyrogallol, reduced glutathione, and dithio-bis(2-nitrobenzoic acid) were from Merck (Kuala Lumpur, Malaysia).

Oil palm (*E. guineensis*) leaves from the Universiti Putra Malaysia plantation were coarsely chopped and dried in a 40°C oven for 24 hours. The dried material was milled and extracted with methanol using a 1:10 (wt/vol) solvent ratio, under continuous agitation (turbo extractor) at room temperature for 2 hours. After filtration, the solvent was completely removed in a rotary evaporator under vacuum at

40°C. The extract was stored in glass jars flushed with nitrogen at −20°C.

Identification of compounds in the extract

The OPLE (80 mg) was hydrolyzed in 6 *M* HCl at 95°C and refluxed for 2 hours in a steam bath. The sample was cooled and filtered through a nylon membrane filter (pore size, 0.4 μm) (Whatman, Maidstone, United Kingdom) before being injected into a reverse-phase high-performance liquid chromatograph on a Symmetry C18 column (150 × 3.9 mm; particle size, 5 μm) (Waters Corp., Milford, MA, USA), using acidified water (trifluoroacetic acid at pH 2.5) and methanol as the mobile phase, on a gradient run, with an ultraviolet detector at 280 nm. Pure standards [(+)-catechin, (−)-epicatechin, epigallocatechin (EGC), EGC gallate (EGCG), epicatechin gallate (ECG), quercetin, myricetin, kaempferol, and rutin] were used as internal and external standards to identify the compounds.

The high-performance liquid chromatography result showed that the main phenolic compounds in OPLE were the green tea catechins, namely, EGC (0.08%), catechin (0.30%), epicatechin (0.01%), EGCG (0.28%), and ECG (0.05%).

Animals

Male Wistar Kyoto (WKY) rats, 16 weeks old, were divided into six groups of eight rats per group: (1) normal control rats; (2) normal rats given OPLE; (3) normal rats given captopril; (4) L-NAME-induced NO-deficient rats given vehicle; (5) L-NAME-induced NO-deficient rats given OPLE; and (6) L-NAME-induced NO-deficient rats given captopril. The rats were maintained on distilled water and a standard rat chow (Gold Coin Sdn. Bhd., Klang, Malaysia) for 14 days and kept in a well-ventilated room with a 12-hour dark/light cycle before the study. Measures were taken to minimize pain or discomfort, and experiments were carried out in accordance with the animal ethics approval of the Universiti Putra Malaysia.

Soy oil was used as the vehicle for dissolving and dispensing OPLE (500 mg of OPLE/kg/day by oral gavage) at a concentration of 100 mg/mL. The dose of 500 mg/kg was used in accordance with previous studies on green tea catechins[15] and shown to be nontoxic to rats.[16] Water-soluble L-NAME (60 mg/L) was administered in the drinking water. Water-soluble captopril (100 mg/kg/day) was coadministered with L-NAME in the drinking water as the positive standard drug. The vehicle was administered in all rats at similar amounts and with a similar method to rats receiving OPLE.

Blood samples (2 mL) from nonfasting rats were obtained in the morning via cardiac puncture with the animal under ketamine/xylazine (ketamine, 50 mg/kg; xylazine, 5 mg/kg) combination anesthesia, at weeks 0, 6, 9, and 12. Heparinized tubes (for plasma) and tubes with no additive (for serum) were used. The heparinized blood was kept on ice, and the plasma was recovered by centrifugation at 3,000 *g* at 4°C for 15 minutes. Erythrocytes were washed three times with an equal volume of saline and centrifuged at 3,000 *g* for 15 minutes in between washings. Aliquots of the washed erythrocytes and plasma were frozen at −80°C until analyzed. Blood collected with no-additive tubes was incubated at 37°C and left to fully clot for 90 minutes, after which it was centrifuged at 3,000 *g* for 15 minutes. An aliquot of the resultant serum was collected and frozen at −80°C until assayed.

Blood pressure

Indirect systolic blood pressure (BP) was measured in rats within 10 minutes of immobilization, under mild intramuscular ketamine/xylazine anesthesia (a dose similar to those used during blood sampling) using noninvasive tail cuff BP plethysmography (Powerlab system for data handling, ADInstruments, Sydney, Australia). BP was taken before noon, and for each rat values were averaged from five readings. A preliminary study confirmed that BP values measured within 10 minutes of immobilization in ketamine/xylazine-anesthetized rats were similar to that of conscious rats.[17] Diastolic BP was not measured because the noninvasive BP is limited to only measuring systolic BP.

Antioxidant activities

Serum NO was measured using a standard method (modified Griess reagent for NO_2/NO_3 assay) and measuring absorbance at 540 nm with a microplate reader (Thermo Labsystems, Beverly, MA, USA) after 15 minutes. Sodium nitrite (5–100 ppm) solutions were used for calibrating the nitrite concentration.

The antioxidant enzyme activities in tissues were determined. For superoxide dismutase (SOD) activities, the absorbances of 500 μL of hemolysate (1 part packed erythrocytes to 3 parts ice-cold deionized water) and the average of four preparations of blank control were used to calculate the enzyme unit, which is the amount that inhibits 50% pyrogallol reaction.[18]

For catalase activities, 1 part packed erythrocytes to 4 parts ice-cold deionized water were mixed and then diluted 500 times with 50 m*M* pH 7.0 phosphate buffer. Two milliliters of this was added to 1 mL of 30 m*M* hydrogen peroxide (H_2O_2), and the decrease in absorbance was followed for 30 seconds.[19] The H_2O_2 enzymatic decomposition follows a first-order reaction, and the rate constant (k) is a direct measure of the catalase concentration.

For glutathione peroxidase (GSH-Px) activities, aliquots of 400 μL of hemolysate consisting of 1 part packed erythrocytes mixed with 3 parts ice-cold deionized water were used.[20] One unit of enzyme activity was the decrease in the log reduced glutathione concentration of 0.001/minute after subtracting the decrease in log reduced glutathione concentration per minute for the nonenzymatic reaction.

Plasma malondialdehyde (MDA) was determined according to standard proceedures,[21] where plasma (300 μL) was mixed with 42 m*M* H_2SO_4 (2.4 mL) and 10% aqueous sodium tungstate (300 μL) and vortex-mixed. After 10 minutes, the mixture was centrifuged at 3,000 *g* for

10 minutes. The pellet was resuspended in 450 μL of distilled water and then vortex-mixed until homogeneous. Fifty microliters of 7 m*M* *tert*-butylhydroxytoluene (in 50% ethanol solution), 3.0 mL of 0.05 *M* HCl, and 1.0 mL of 1% aqueous thiobarbituric acid solution were added, mixed thoroughly, heated immediately for 60 minutes at 95°C, and cooled with running water. *n*-Butanol (4 mL) was then added and vortex-mixed for 60 seconds, after which the mixture was centrifuged for 10 minutes at 3,000 *g*. The absorbance of the organic layer was read at 532 nm against pure *n*-butanol,[21] using a Secoman (Anthelie, Domont, France) Advance UV-visible spectrophotometer.

Histology study

Medial transverse sections of the hearts were fixed in 10% neutral buffered formalin for histology studies. The remaining heart was immediately frozen at −80°C until analyzed. For the antioxidant enzyme and MDA analysis, the frozen tissue was thawed overnight at 4–8°C and minced with surgical scissors before being homogenized in ice-cold 0.15 *M* KCl (4 mL for every 1 g of tissue) using a Silverson (Chesham, United Kingdom) tabletop homogenizer set (model L4RT). The homogenate was freed from cellular debris and nuclei by centrifugation at 5,000 *g* at 4°C for 20 minutes. The resultant supernatant was used for the biochemical assays. Homogenate protein content was determined and calibrated with bovine serum albumin.[22] Organ somatic index was measured as (organ mass/body mass) × 100.

Five-micrometer-thick tissue sections were deparaffinized and processed routinely for hematoxylin and eosin staining. Approximately 10 randomly selected fields of each heart section were observed with a light microscope at ×100 magnification. The occurrence of myocardial fibrosis was examined in each heart section, and the percentage was estimated for every field. Myocardium and coronary arteriole wall thicknesses and lumen diameter were measured with a ruler placed on the images captured at ×400 magnification on a flat computer monitor. Five randomly selected images of the myocardium and a minimum of two images of the coronary arterioles from each heart section were used for myocardial thickness and arterial measurements, respectively. The myocardial fibers thicknesses (in mm) were measured from the computer screen, solely for comparison purposes. Wall-to-lumen ratios were calculated as (wall thickness/lumen diameter) × 100. All images were captured using a PixeLINK version 1.3 megapixel camera (PixeLINK, Ottawa, Canada) mounted on a Leica DME light microscope (Leica Microsystems, Buffalo Grove, IL, USA).

All statistical analyses were performed using Minitab (State College, PA, USA) version 13 statistical software, and data were expressed as mean ± SEM values. Significant differences ($P < .05$) between groups were analyzed using a one-way analysis of variance, followed by Tukey's pairwise comparison *post hoc* test.

RESULTS

The OPLE catechins were tested on normal and hypertensive WKY rats. The average fluid intake per rat per day was significantly higher compared with baseline in the captopril and L-NAME groups (Table 1). The hypertensive rats (L-NAME) showed significantly ($P < .05$) lower weight gains by about 30% compared with normal rats (Table 2). Captopril treatment of normal or hypertensive rats reduced this weight gain further. The cardiosomatic index in L-NAME and L-NAME + OPLE rats indicated about 20% hypertrophy (Table 2) compared with the normotensive control rats. Captopril prevented hypertrophy in L-NAME rats.

L-NAME gradually increased the WKY rats' BP from 100–110 mm Hg to 140.00 ± 4.18 mm Hg within 1 week and to about 175 mm Hg from week 2 onward. Co-administration of L-NAME with OPLE or captopril significantly attenuated this BP increase to normal values (Fig. 1) throughout the experimental duration. OPLE and captopril caused no hypotensive or other apparent toxic effects in normotensive rats. The BP in all normotensive rats was

Table 1. Effects of Chronic Treatments on Fluid and Food Intake of Rats

	Average water intake (mL/rat/day)			*Average food intake (g/rat/day)*		
Group	*Week 0*	*Weeks 1–6*	*Weeks 7–12*	*Week 0*	*Weeks 1–6*	*Weeks 7–12*
WKY						
Control	35 ± 2	33 ± 2	36 ± 2	14 ± 1	13 ± 2	16 ± 5
+OPLE	38 ± 2	35 ± 3	38 ± 2	14 ± 2	14 ± 1	16 ± 2
+captopril	38 ± 2	53 ± 2*[a]	56 ± 2*[a]	15 ± 1	15 ± 2	17 ± 5
+L-NAME	35 ± 4	40 ± 3	41 ± 3*[a]	14 ± 1	13 ± 2	16 ± 5
+L-NAME + OPLE	38 ± 3	49 ± 2*[a]	45 ± 3*[a]	14 ± 2	14 ± 1	16 ± 2
+L-NAME + captopril	34 ± 2	48 ± 3*[a]	48 ± 3*[a]	15 ± 1	15 ± 2	17 ± 5
SHR						
Control	34 ± 7	34 ± 3	33 ± 3	21 ± 1[a]	24 ± 2[a]	17 ± 2
+OPLE	32 ± 3	32 ± 6	33 ± 6	18 ± 3	20 ± 2[a]	20 ± 4[a]
+captopril	40 ± 9	48 ± 2[a]	39 ± 4	20 ± 4[a]	25 ± 2[a]	20 ± 2[a]

Data are mean ± SEM values.

[a]$P < .05$ versus Wistar–Kyoto (WKY) control; *$P < .05$ versus week 0.

L-NAME, N^{ω}-nitro-L-arginine methyl ester; OPLE, oil palm (*E. guineensis*) leaf extract; SHR, spontaneously hypertensive.

Table 2. Effects of Chronic Treatments on Final Body Weight and Final Heart Weight of Rats

Group	*Final heart weight (g)*	*Cardiosomatic index (mg/100 g)*	*Final body weight (g)*	*Death*	*Final* n	*Weight gain (g)*	*% body weight gain*
WKY							
Control	1.22 ± 0.06	3.7 ± 0.2	333 ± 7	0	8	89 ± 7	35
+OPLE	1.26 ± 0.10	3.9 ± 0.3	325 ± 1	0	8	79 ± 7	32
+captopril	1.02 ± 0.04	3.6 ± 0.2	291 ± 9	0	8	58 ± 7[a]	20[a]
+L-NAME	1.45 ± 0.19	4.5 ± 0.7	334 ± 14	5	3	27 ± 3[a]	9[a]
+L-NAME + OPLE	1.48 ± 0.11	4.8 ± 0.3[a]	311 ± 24	2	6	21 ± 2[a]	8[a]
+L-NAME + captopril	0.91 ± 0.07	3.1 ± 0.3[b]	296 ± 6	1	7	16 ± 2[a]	6[a]
SHR							
Control	1.20 ± 0.09	4.5 ± 0.3	267 ± 6[a]	0	8	29 ± 3[a]	12[a]
+OPLE	0.93 ± 0.10	3.5 ± 0.4[ac]	266 ± 5[ac]	0	8	39 ± 3[ac]	17[ac]
+captopril	1.16 ± 0.16	4.5 ± 0.6	258 ± 7[a]	0	8	19 ± 2[ac]	8[ac]

Data are mean ± SEM values.
[a] $P < .05$ versus WKY control; [b] $P < .05$ versus WKY + L-NAME; [c] $P < .05$ versus SHR control; * $P < .05$ versus week 0.

unchanged throughout the study. During the course of the study eight animals died: five from the L-NAME group (one each at weeks 6, 8, 10, 11, and 12), two from the L-NAME + OPLE group (at weeks 8 and 9), and one from the L-NAME + captopril group (at week 7). Three of the animals from the L-NAME group died between weeks 10 and 12, and postmortem examination showed the deaths were due to respiratory failure as a result of pulmonary edema, associated with hypertension-induced cardiac failure (cor pulmonale); the other two rats died of unknown causes at weeks 6 and 8. The rats that died from the L-NAME + captopril and L-NAME + OPLE groups were also deaths from unknown causes because they died at night and the carcasses were already degraded when found the following morning.

Generally, MDA levels increased with age, but the net increases were lowest ($P < .05$) in the OPLE-treated hypertensive conditions (L-NAME + OPLE), pointing to the an-

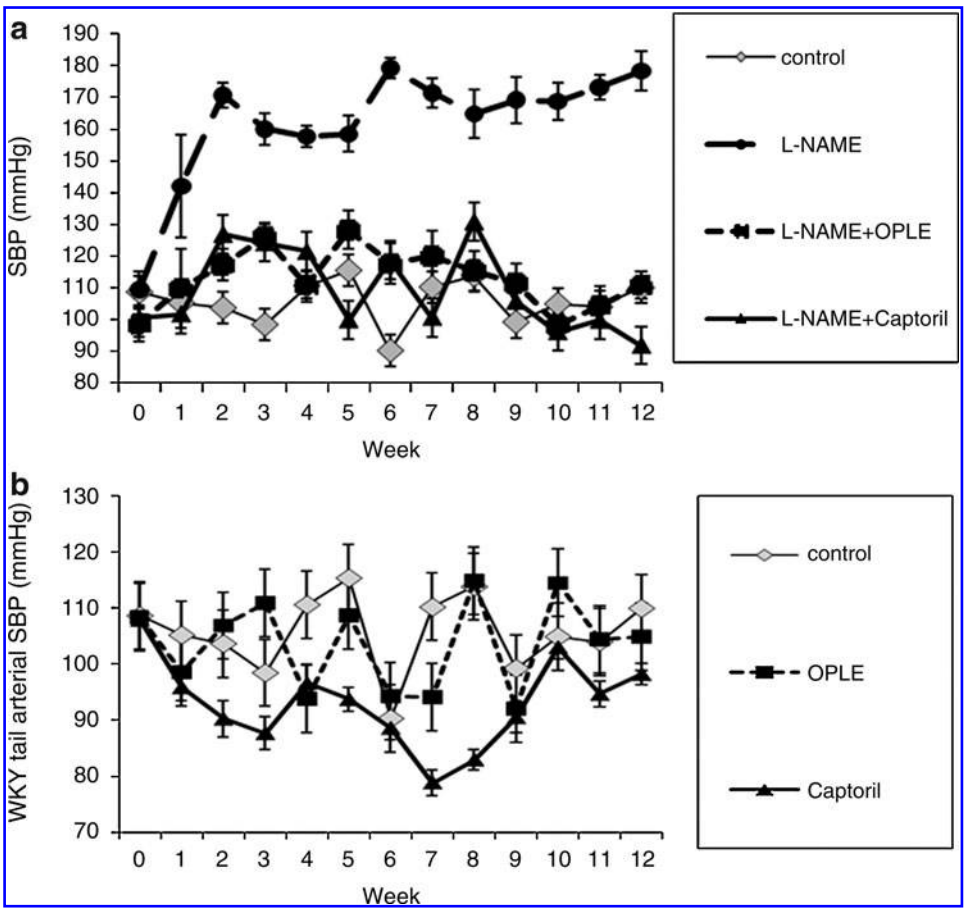

FIG. 1. Effect of chronic treatments with OPLE and captopril on systolic blood pressure (SBP) of (**a**) L-NAME-treated and (**b**) normal WKY rats. Data are mean ± SEM values.

Table 3. Plasma Malondialdehyde Concentration of Experimental Groups for the Period of Study

WKY group	Plasma MDA concentration (μmol/L)			Net MDA increase (μmol/L) (week 12 – week 0)
	Week 0	Week 6	Week 12	
Control	5.85 ± 0.18	7.06 ± 0.09	8.74 ± 0.67*	2.89 ± 0.34
+OPLE	5.30 ± 0.32	5.62 ± 0.61	7.50 ± 0.51	2.20 ± 0.46
+captopril	5.67 ± 0.99	6.36 ± 0.03	7.83 ± 0.49	2.16 ± 0.51
+L-NAME	4.66 ± 0.86	8.35 ± 0.49	8.43 ± 0.84*	3.77 ± 0.73
+L-NAME + OPLE	6.57 ± 0.43	6.59 ± 0.36[b]	7.17 ± 0.85	1.13 ± 0.52[ab]
+L-NAME + captopril	5.44 ± 0.28	6.80 ± 0.38	8.29 ± 0.73*	2.85 ± 0.47

Data are mean ± SEM values.
[a]$P < .05$ versus WKY control; [b]$P < .05$ versus WKY + L-NAME; *$P < .05$ versus week 0.
MDA, malondialdehyde.

tioxidative properties of OPLE. Net MDA increases were highest ($P < .05$) in the L-NAME groups ($\sim$30% higher than normal). The OPLE treatment (but not captopril) reduced this MDA increase ($P < .05$) by 70% compared with untreated L-NAME rats, indicating OPLE's *in vivo* antioxidative effects (Table 3). Aging caused activities of endogenous antioxidant enzymes (erythrocyte SOD, catalase, and GSH-Px) to increase (Table 4). However, under hypertensive conditions, the reverse was observed for erythrocyte SOD. The OPLE did not affect the SOD activities in all groups. Increases in catalase activities were only significant ($P < .05$) in the untreated normal and L-NAME + OPLE rats at week 12. The GSH-Px activity increases were significant ($P < .05$) in the untreated hypertensive rats, and both the OPLE and captopril treatments reversed this trend in L-NAME rats. The temporary changes in activities of antioxidant enzymes at week 6 (slight reduction in GSH-Px activities for most untreated rat groups and significant [$P < .05$] catalase increase in L-NAME groups) may have coincided with sexual maturity.

Chronic L-NAME treatment significantly ($P < .05$) decreased serum NO levels by 80% (week 6), and captopril significantly ($P < .001$) maintained the serum NO at normal levels (Table 5). However, by week 9, the serum NO level in both the L-NAME and L-NAME + OPLE groups had returned to near normal values, whereas the serum NO level in L-NAME + captopril rats remained significantly higher than that of untreated L-NAME rats ($P < .01$). The OPLE treatment gradually increased serum NO levels of L-NAME rats, such that by week 12 they were significantly higher than in the untreated L-NAME rats ($P < .05$) and similar to those in normotensive WKY rats. In contrast, the serum NO level decreased in L-NAME + captopril rats to baseline levels. The serum NO level remained unchanged with age in most groups during the study except in L-NAME rats ($P < .05$), which showed a transient reduction at week 6.

Heart

The OPLE treatment significantly reduced MDA ($P < .05$) and SOD ($P < .05$) activities in NO-deficient rats' hearts compared with control and L-NAME rats, respectively (Table 6). No significant differences in catalase and GSH-Px activities were found among all groups.

Myocardial fibrosis was observed as pink scar patches on the heart sections, with occasional infiltration with inflammatory cells (Fig. 2c). The NO deficiency dramatically increased the fibrosis to 60% compared with control rats ($P < .001$). OPLE or captopril co-administration could not reduce this damage in L-NAME rats (Table 6). The muscle fibers of NO-deficient rats were noticeably thicker,

Table 4. Activities of Antioxidant Enzymes in Erythrocytes of Experimental Groups for the Period of Study

WKY group	Week 0	Week 6	Week 9	Week 12
SOD (mU/mL)				
Control	13.0 ± 0.8	22.8 ± 1.1[a]	16.2 ± 01.4	20.1 ± 0.7[a]
+OPLE	14.6 ± 1.2	22.7 ± 1.0[a]	18.0 ± 0.7	19.5 ± 1.0[a]
+captopril	14.0 ± 1.0	21.8 ± 0.6[a]	14.8 ± 1.9	18.6 ± 0.6[a]
+L-NAME	13.8 ± 0.9	12.0 ± 0.9[b]	08.1 ± 0.4[ab]	10.3 ± 1.2[b]
+L-NAME + OPLE	12.7 ± 1.4	09.9 ± 0.7[b]	06.5 ± 0.9[ab]	10.7 ± 0.8[b]
+L-NAME + captopril	13.3 ± 1.0	12.6 ± 1.1[b]	07.2 ± 0.8[ab]	10.8 ± 0.8[b]
Catalase (mU/mL)				
Control	11.4 ± 0.9	13.6 ± 1.1	12.8 ± 0.4	13.9 ± 0.5[a]
+OPLE	13.2 ± 0.7	13.6 ± 1.3	15.6 ± 0.7	17.6 ± 0.8
+captopril	13.5 ± 0.6	13.4 ± 0.3	14.0 ± 0.5	13.1 ± 0.8
+L-NAME	11.8 ± 0.7	15.0 ± 0.7[a]	11.5 ± 0.6	13.8 ± 0.5
+L-NAME + OPLE	12.4 ± 0.4	12.9 ± 0.6	12.6 ± 0.7	15.4 ± 0.9[a]
+L-NAME + captopril	12.9 ± 0.5	12.5 ± 0.5	13.1 ± 0.6	14.3 ± 0.3
GSH-Px (U/mL)				
Control	19.8 ± 3.8	18.8 ± 2.5	20.0 ± 5.8	24.3 ± 4.2
+OPLE	20.7 ± 3.2	25.2 ± 3.8	22.6 ± 4.9	27.0 ± 3.2
+captopril	16.5 ± 0. 9	21.1 ± 5.1	14.1 ± 1.8	26.2 ± 4.4
+L-NAME	22.6 ± 1.8	10.0 ± 1.3[a]	15.9 ± 1.8	31.2 ± 3.2[a]
+L-NAME + OPLE	21.4 ± 5.7	14.0 ± 4.0	16.5 ± 1.7	18.9 ± 1.9
+L-NAME + captopril	22.1 ± 3.1	14.2 ± 2.0	16.6 ± 2.7	17.2 ± 0.9

Data are mean ± SEM values.
[a]$P < .05$ versus week 0 (for the respective group); [b]$P < .05$ versus normotensive WKY control.
GSH-Px, glutathione peroxidase; SOD, superoxide dismutase.

Table 5. Serum Nitric Oxide Values of Experimental Groups for the Period of Study

	Nitric oxide (ppm)			
WKY group	*Week 0*	*Week 6*	*Week 9*	*Week 12*
Control	0.414 ± 0.024	0.548 ± 0.009	0.417 ± 0.053	0.384 ± 0.038
+OPLE	0.347 ± 0.014	0.409 ± 0.109	0.468 ± 0.096	0.362 ± 0.002
+captopril	0.381 ± 0.023	0.573 ± 0.024	0.455 ± 0.060	0.525 ± 0.071
+L-NAME	0.339 ± 0.034	0.104 ± 0.056[ab]	0.276 ± 0.061	0.210 ± 0.074
+L-NAME + OPLE	0.319 ± 0.044	0.197 ± 0.053[b]	0.283 ± 0.084	0.531 ± 0.086[c]
+L-NAME + captopril	0.402 ± 0.008	0.585 ± 0.082[c]	0.742 ± 0.123[bc]	0.44 ± 0.072

Data are mean ± SEM values.
[a]$P < .05$ versus week 0 (for the respective group); [b]$P < .05$ versus normotensive WKY control within the same week; [c]$P < .05$ versus L-NAME.

indicating hypertrophy ($P < .001$). Unlike the L-NAME + OPLE group, the L-NAME + captopril group demonstrated near-normal myocardium, with no increase in thickness.

Changes in coronary small arteries

The coronary artery walls were noticeably thickened after 12 weeks of NO deficiency (Table 6) with significant changes in the wall-to-lumen ratio compared with control rats ($P < .005$). There were decreases in artery wall thickness with concomitant administration of OPLE or captopril resulting in decreased wall-to-lumen ratios to normal values (Table 6). The aorta wall thickness and coronary artery wall-to-lumen ratio of all normotensive WKY rats were similar, and neither OPLE nor captopril affected them.

DISCUSSION

BP effects

Phenolic compounds purportedly enhance vascular NO activities, either by inducing NO production through NO synthase expression or by their direct antioxidant effect, thereby protecting NO against oxidative destruction.[23] L-NAME inhibits NO synthesis, impairing endothelial-dependent vasodilation, and additionally induces hypertension via excess O_2^- formation, from the vascular smooth muscle layer,[23] and contributes to the reduced serum NO in these rats (Table 5), whereas O_2^- accelerated the degradation of NO to peroxynitrite.[24] The L-NAME-induced hypertension would cause significant angiotensin converting enzyme activity elevation in the heart and aorta after 4 weeks.[25] Overproduction of local angiotensin II would thus lead to increased vascular O_2^- formation through the expression of NADPH-dependent oxidase in aortic smooth muscle cells.[26] Captopril inhibits angiotensin converting enzyme, increases the NO/O_2^- ratio, reduces NADPH oxidase activity resulting in normalized BP,[27] and normalizes serum NO (Table 5). The NADPH oxidase and the endothelial NO synthase are the major sources of superoxides in the endothelial cells.[28] The catechin-rich OPLE normalizes BP in NO-deficient rats, most likely by quenching the superoxides, resulting in BP effects similar to that of captopril although probably via different mechanisms. More important is that OPLE did not alter the BP of normotensive rats.

Cardiovascular tissue effects

The cardiosomatic index is a gross indicator of left ventricle hypertrophy. The cardiosomatic indices for the L-NAME and L-NAME + OPLE groups were not significantly different from each other, but that of L-NAME + OPLE was higher than that of normotensive rats. However, both groups were beginning to develop left ventricle hypertrophy, associated with an increased protein synthesis in cardiac myocytes and increased myocardial fibrosis. In contrast, L-NAME + captopril rats displayed reduced cardiosomatic index and myocardial fiber thickness, although the degrees of fibrosis were similar to those of the L-NAME group.

The catechin-rich OPLE was able to inhibit the L-NAME-induced NO-deficiency hypertension, but not the associated

Table 6. Superoxide Dismutase, Catalase, and Glutathione Peroxidase Activities and Malondialdehyde Levels in Hearts of Experimental Rats

WKY group	*MDA (nmol/g of tissue)*	*SOD (units/mg of protein)*	*Catalase (*k*/mg of protein)*	*GSH-Px (units/mg of protein)*
Control	23.49 ± 2.17	3.91 ± 0.07	4.39 ± 0.56	3.35 ± 0.71
+OPLE	18.77 ± 1.99	3.69 ± 0.11	6.08 ± 1.02	4.16 ± 0.71
+captopril	17.54 ± 0.41	3.91 ± 0.004	5.42 ± 1.02	5.20 ± 1.14
+L-NAME	18.73 ± 3.17	4.54 ± 0.80	5.02 ± 0.99	5.33 ± 0.48
+L-NAME + OPLE	13.01 ± 1.20[a]	3.25 ± 0.21[b]	4.11 ± 0.53	4.69 ± 0.44
+L-NAME + captopril	18.31 ± 2.05	3.64 ± 0.36	4.31 ± 0.78	4.84 ± 0.36

Data are mean ± SEM values ($n = 8$).
[a]$P < .05$ versus WKY control; [b]$P < .05$ versus WKY + L-NAME.

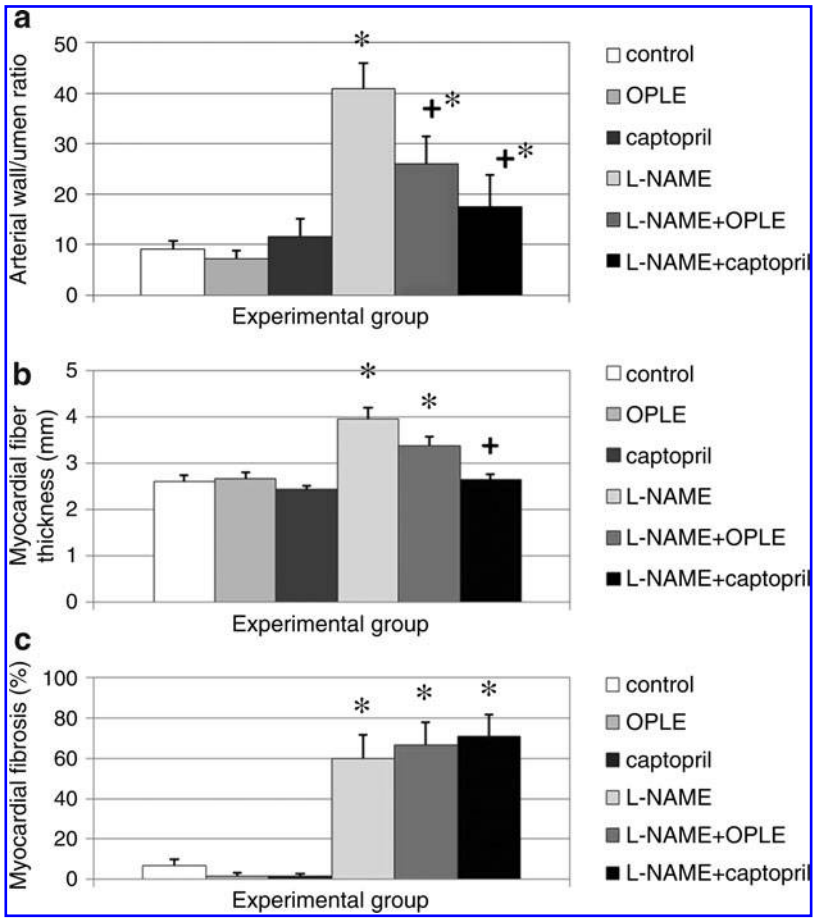

FIG. 2. Effect of chronic treatments with OPLE and captopril in normal and nitric oxide–deficient WKY rats on (**a**) coronary arteriole wall-to-lumen ratio, (**b**) myocardial fiber thickness, and (**c**) percentage of myocardial fibrosis. $^{*}P < .05$ versus control, $^{+}P < .05$ versus L-NAME.

cardiac hypertrophy. Similarly, red wine polyphenols reportedly reduced BP, increased NO synthase activity, and prevented myocardial fibrosis in L-NAME rats without affecting left ventricle hypertrophy.[28] Cardiac hypertrophy in L-NAME-treated animals is remedial to chronic hypertension and is associated with plasma renin activity increases.[28] Plasma renin is higher in animals treated with lower doses than with greater doses of L-NAME.[28] This may explain the cardiac hypertrophy in the L-NAME + OPLE group. In contrast, green tea extract, another rich source of catechin, successfully prevented BP increases and attenuated cardiac hypertrophy in an angiotensin II rat model of hypertension, probably because of the high dose (1 g/kg/day) of green tea extract used.[29]

Hypertension causes coronary artery narrowing, due to arterial structural changes to withstand the increasing pressure on the arterial wall. The increased wall-to-lumen ratio indicated remodeling of the small arterial wall. With OPLE treatment, vascular remodeling was reduced as the wall-to-lumen ratio was not as extensive as in the untreated L-NAME rats. The attenuation in BP increases by OPLE apparently minimizes artery structural changes. In OPLE-treated normal rats, the normal appearance of myocardium and unchanged antioxidant status confirmed that OPLE was not cardiotoxic. The catechins have enzyme and cell signaling modulating properties,[30] thus providing some protection and reducing certain metabolic dysfunctions.

Antioxidant effects

The L-NAME treatment increased MDA (a lipid peroxidation product) levels (by 80%) and reduced erythrocyte SOD and GSH-Px activities in week 6, compared with normal rats. This increased oxidation inactivated GSH-Px, resulting in an enhanced H_2O_2 level, which in turn inactivated SOD.[31] By week 12 the enzymes had been upregulated, in response to the oxidative stress. Catalase activities slightly increased with age but remained stable when compared among groups at any week. The catechin-rich OPLE gradually improved serum NO levels in L-NAME rats and significantly by 12 weeks. The OPLE significantly reduced plasma MDA content in L-NAME rats, confirming its antioxidative abilities to reduce oxidative stress.

Other investigators showed that (–)-epicatechin can improve BP in hypertensive patients, decrease infarct size in myocardial ischemia–reperfusion injury animal models, and reduce myocardial infarction size and left ventricular scar area strains.[32] Recent reports indicate that (–)-epicatechin exerted cardioprotective actions, partly via endothelial NO synthase–mediated NO production in endothelial cells. The

(–)-epicatechin apparently only partially stimulated NO production in cells, involving a cell surface acceptor–effector. (–)-Epicatechin treatment activates endothelial NO synthase via $Ser^{633}$and Ser^{1177} phosphorylation, Thr^{495} dephosphorylation, and the phosphatidylinositol 3-kinase pathway and at least partially mediated via the Ca^{2+}/calmodulin kinase II pathway.[33] Epidemiological studies consistently associate high flavonoid intake with a reduced risk of cardiovascular diseases, linked to nonspecific antioxidant and anti-inflammatory properties. An increasing body of evidence suggests that flavonoids specifically target molecular structures including cardiovascular ion channels, important in vascular tone regulation and cardiac electric activity, thus inducing antihypertensive and cardioprotective effects.[34]

Hypertension and cardiovascular disease are associated with hyperglycemia and dyslipidemia, low-grade inflammation, and serum NO-linked insulin resistance. Other animal models have been used to study these effects.[35] The protective mechanisms of dietary flavonoids and green tea catechins against cardiovascular disease risk include through (1) antioxidant actions, (2) central nervous system effects, (3) intestinal transport alterations, (4) fatty acid sequestration and processing, (5) peroxisome proliferator-activated receptor activation, (6) increased insulin sensitivity,[36] (7) maintaining endothelial functions, vascular homeostasis, and decreased atherogenesis,[37] (8) vascular NO production,[38] and (9) inflammatory and endothelial apoptosis modulation.[39] EGCG was reported to help prevent cardiac hypertrophy and hypertension by suppressing (1) angiotensin II and pressure overload, (2) reactive oxygen species generation and NADPH oxidase overexpressions, (3) nuclear factor-κB and activator protein-1 activation, (4) reactive oxygen species–dependent p38 and c-Jun N-terminal kinase signaling pathways, (5) epidermal growth factor receptor transactivation, (6) extracellular signal-regulated kinases/phosphatidylinositol 3-kinase/Akt/mTOR/p70 (S6K), and (7) reactivation of atrial natriuretic peptide and brain natriuretic peptide and is involved in inhibition of various intracellular signaling transductional pathways.[40] Epicatechin and its procyanidin oligomers inhibit angiotensin converting enzyme activity, with the tetramer being the most active inhibitor.[41] EGCG but not EGC apparently inhibited the angiotensin II-stimulated (1) vascular smooth muscle cell hypertrophy and (2) c-Jun N-terminal kinase signaling pathway at both the transcriptional and post-translational levels, but did not change extracellular signal-regulated kinase and p38 mitogen-activated protein kinase.[42] Future investigation to measure inducible NOS and other enzyme or signaling activities may help confirm the mechanisms of OPLE catechins.

CONCLUSIONS

This is the first report on the *in vivo* antihypertensive properties of catechins extracted from an alternative source, namely, oil palm leaf, for use as a medicinal food for hypertension and other cardiovascular ailments. The OPLE catechins alleviated NO-deficiency hypertension by (1) normalizing serum NO levels, (2) producing antioxidant effects, and (3) enhancing endothelial NO bioactivity possibly via O_2^- scavenging ability in hypertensive rats. The OPLE catechins showed some cardioprotective effects by reducing coronary arteriole wall thickening induced by NO deficiency and showed no chronic toxicity to the cardiovascular system.

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AUTHOR DISCLOSURE STATEMENT

Currently the authors have no competing financial interests in this research but may wish to commercialize the research through the university in the future (a patent related to this has been filed).

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