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Effects of catechin-rich oil palm leaf extract on normal and hypertensive rats' kidney and liver

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ABSTRACT

Catechin-rich oil palm (*Elaeis guineensis)* leaf extract (OPLE) possesses good ex vivo vasodilation, antioxidant and cardiovascular properties. This study evaluated the beneficial or toxic effects of OPLE on the liver and kidneys of normal and hypertensive rats. The OPLE (500 mg/kg body weight) were administered orally to normal Wistar Kyoto rats, spontaneously hypertensive rats (SHR) and *N*-ω-nitro-L-arginine methyl ester (L-NAME)-induced NO-deficient hypertensive rats. The OPLE reduced hypertension in NO-deficient rats, but not in SHR. Hepatocytes or glomeruli injury and oxidative markers were high in hypertensive rats compared to normal rats, and they were reduced ($p < 0.05$) by OPLE supplementation, even when there was no blood pressure reduction. Unlike the hypertensive drug captopril, the OPLE showed no toxicity to normal rats. The dose reported is equivalent 0.5 g of catechins/day for humans or 2.5 cups of tea. The catechins are from an abundant alternative source for potential use as functional food.

1. Introduction

The liver and kidney actively detoxify and handle endogenous and exogenous chemicals, making them vulnerable to injury. Palm leaves are abundant, under-utilised, by-products of the palm oil industry in tropical countries like Indonesia, Thailand, Malaysia, Africa and South America. The palm leaves have been used for decades as ruminant feed without any reports of toxicity. The oil palm (*Elaeis guineensis)* leaves methanolic extract (OPLE) are rich in flavonoids and green tea catechins (Jaffri et al., 2011). The OPLE decreased coronary arteriole wall-lumen ratio to near normal values, and showed good cardio-protective effects under NO deficiency (Jaffri et al., 2011). The OPLE is a potential economically viable, new source of green tea catechins. The health benefits of green tea catechins for the prevention of cancer, inflammation, arthritis, bacteria, angiogenesis, oxidative stress, virus, neurodegeneration and hypercholesterol, had been documented (Naghma & Hasan, 2007). However, the absence of reports on the toxic or beneficial effects of catechin-rich oil palm leaves extract on the liver and kidney *in vivo*, requires investigation and confirmation.

The catechin-rich OPLE showed vasodilative properties on noradrenaline preconstricted rat aorta and mesenteric arterial bed (Abeywardena, Runnie, Salleh, Mohamed, & Head, 2002). The OPLE also retarded low density lipoprotein (LDL) oxidation better than other edible plant extracts (Salleh, Runnie, Roach, Mohamed, & Abeywardena, 2002). Microvascular changes lead to alterations in organ perfusion and causes hypertension-related liver and kidney damages. Hypertension is a major risk factor that predisposes the liver and kidney to disorders. The administration of *N*-ω-nitro-L-arginine methyl ester (L-NAME), which is a non-selective nitric oxide synthase (NOS) inhibitor, induces NO-deficiency, hypertension and increases oxidative stress. Dietary L-NAME increase microvascular pressure in the liver and kidney *in vivo* and *in vitro* (Mittal, Gupta, Lee, Sieber, & Groszmann, 1994). This NO-deficiency model together with spontaneously hypertensive rats (SHR) are useful tools for the study of natural products effects (toxic or protective) under hypertension and oxidative stress. The discovery of the OPLE ex vivo vasodilatory properties instigated this investigation on the *in vivo* liver and kidney effects of OPLE in SHR and NO-deficient hypertensive rats.

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2. Materials and methods

2.1. General

Pyrogallol, reduced glutathione (GSH) and dithio-bis(2-nitrobenzoic acid) (DTNB) were purchased from Merck (Malaysia). Captopril, L-NAME, thiobarbituric acid (TBA) and 1,1,3,3-tetraethoxypropane (TEP) were purchased from Sigma–Aldrich (Malaysia). All chemicals used were of analytical grade. Soy oil was purchased from the local supermarket.

Oil palm (*E. guineensis)* leaves (fronds) collected from the Universiti Putra Malaysia (UPM) plantation, were cut into small pieces and dried in an oven at 40 °C for 24 h. The dried material was then milled to a powder (16 kg), and extracted with methanol at a 1:10 (w/v) solvent ratio, under continuous agitation in a turbo extractor at room temperature for 2 h. After filtration into a rotary evaporator, the solvent was completely removed under vacuum at 40 °C, to yield a dark green waxy material. The extract was stored in glass jars, flushed with nitrogen and kept at −20 °C.

2.2. Compounds identification of OPLE

The OPLE (80 mg) were hydrolysed at 95 °C in 6 M HCl and refluxed for 2 h on a steam bath. The sample was cooled and filtered on a 0.4 μm (Whatman) nylon membrane filter before injected into a RP-HPLC on a Symmetry C18 column 150 × 3.9 mm, 5 μm, (Waters Milford, MA, USA), using acidified water (TFA at pH 2.5) and methanol as mobile phase, on a gradient run, with a UV detector at 280 nm. Pure standards (e.g. (+)-catechin, (−)-epicatechin; epigallocatechin (EGC), epigallocatechin gallate (EGCG), epicatechin gallate (ECG), quercetin, myricetin, kaempferol and rutin were used as internal and external standards to identify the compounds.

2.3. Animal studies

Male Wistar Kyoto (WKY) rats, 16 weeks old, were divided into 6 groups ($N = 8$): (i) Normal control rats; (ii) Normal rats given OPLE; (iii) Normal rats given captopril; (iv) L-NAME-induced NO-deficient rats and vehicle; (v) L-NAME + OPLE; (vi) L-NAME + captopril. Additionally, 21 male spontaneous hypertensive rats (SHR) of 16 weeks old were divided into 3 groups (vii) SHR control; (viii) SHR given OPLE; (ix) SHR given captopril. The rats were acclimatised on distilled water and a standard rat chow (Gold Coin Sdn. Bhd., Klang, Malaysia), for 14 days. They were kept in a well ventilated room with a 12 h dark/light cycle, with unlimited water and food during the study. All experiments were performed according to the guidelines on the ethical use and care of laboratory animals by the Faculty of Veterinary Medicine, University Putra Malaysia and in accordance with the UK Animals Act 1986. Adequate measures were taken to minimise pain or discomfort.

Soy oil was used as the vehicle for dissolving and dispensing OPLE (500 mg OPLE/kg/day by oral gavage) at a concentration of 100 mg/ml. This dose is equivalent to 0.5 g/day for humans, (one 500 mg capsule or 2.5 cups of green tea/day) since rats have a higher metabolic rate. Water soluble L-NAME (60 mg/l) and/or captopril (100 mg/kg/day) were administered in the drinking water. The vehicle was administered in all rats with similar method to rats receiving OPLE.

Blood samples were obtained from unfasted rats via cardiac puncture under ketamine/xylazine anaesthesia (ketamine 50 mg/kg, xylazine 5 mg/kg), using heparinised tubes, at week 0 and 12. Blood was kept on ice and the plasma was recovered by centrifugation at 4000*g* at 4 °C for 15 min. Indirect systolic BP (SBP) was measured in rats that were mildly anesthetized with intramuscular ketamine/xylazine combination (dose similar to those used during blood sampling) within 10 min of immobilisation, using a non-invasive tail cuff BP plethysmography attached to Powerlab system for data handling (AD Instrument, Australia), and Chart 5 software was used for data acquisition. Measurements were taken in the morning before noon for every session. Five readings were measured for each rat and the values were averaged. Preliminary study showed that BP in ketamine/xylazine anesthetized rats was similar to BP in conscious rats within 10 min of immobilisation, as reported by Hatzopoulos, Petruccelli, Laurell, Finesso, and Martini (2002).

At the end of week 12, the rats were sacrificed in random order between 8 am and noon on two consecutive days by an overdose of diethyl ether. The livers and kidneys were excised, weighed and washed with ice cold saline. Hepatosomatic index (HSI) and kidney somatic index (KSI) were measured as the liver or kidney mass/body mass ratio × 100.

The left liver lobes were sliced to approximately 2 mm thickness and immediately fixed in 10% neutral buffered formalin for histology studies. The right kidneys were sliced into 2 halves and the medial transverse sections were similarly fixed. The remaining tissues were immediately frozen at −80 °C for less than four months until use. For the antioxidant enzyme and MDA (a lipid peroxidation marker) analyses, the frozen tissue was thawed overnight at 4–8 °C and minced with surgical scissors, before setting in ice cold 0.15 M KCl (4 ml for every 1 g tissue) and homogenised using a Silverson tabletop homogenizer (model L4RT). The homogenate was freed from cellular debris and nuclei by centrifugation at 5000*g* at 4 °C for 20 min. The resultant supernatant was used for various biochemical assays. The homogenate protein content was determined (Matanjun, Mohamed, Muhammad, & Mustapha, 2010), by calibrating with bovine serum albumin.

The liver enzyme markers, plasma alanine transaminase (ALT), aspartate transaminase (ALT), alkaline phosphatise (ALP) activities, plasma creatinine and blood urea nitrogen (BUN) concentrations were estimated using commercial kits (Roche Diagnostics) for the auto-analyser (Roche/Hitachi, Germany), following the instrument manufacturer's protocol. Creatinine, formed from creatine and excreted by the kidneys was used together with BUN as indicators of changes in glomerular filtration rate.

Five-micrometre tissue sections were deparaffinised and processed routinely for haematoxylin–eosin (H&E) staining. Ten randomly selected fields of each section were observed with a light microscope under low and high power magnification (100× and 400×) and the percentage of abnormal cells, glomerulus and tubules were calculated. Abnormal cells are those which undergo structural changes including swelling, degeneration, fatty changes and cell death.

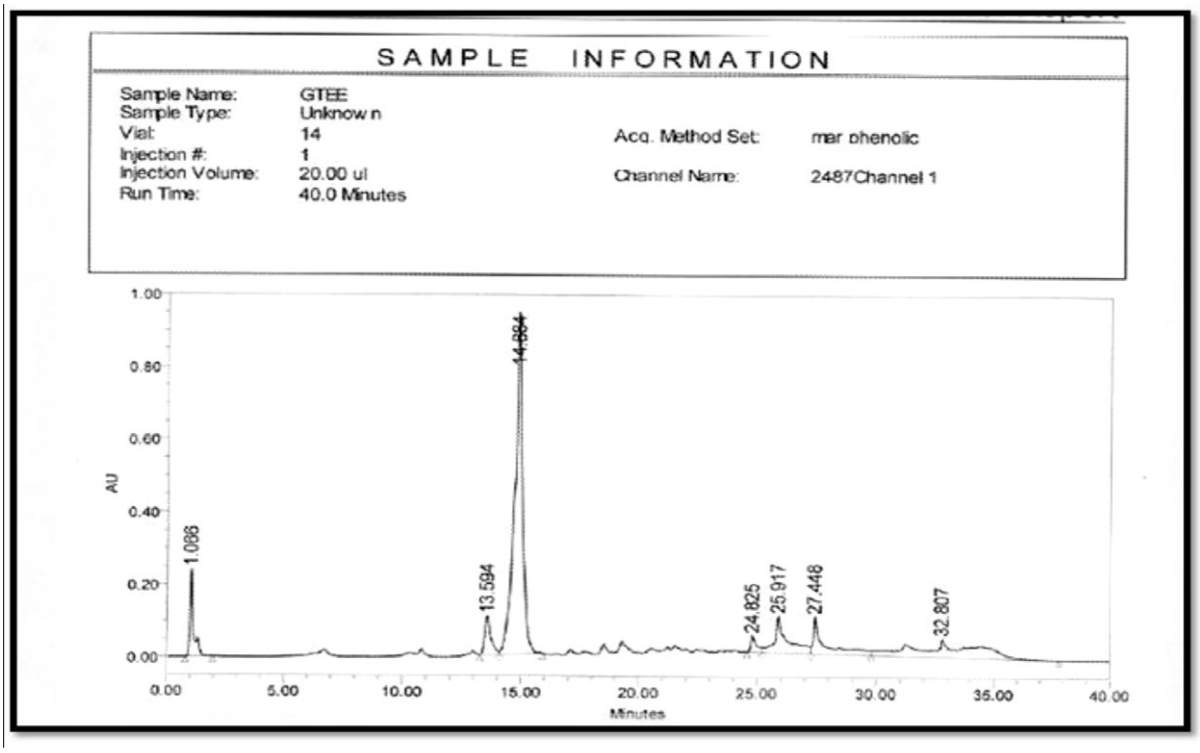
The superoxide dismutase (SOD) activity was determined on 500 μl liver and kidney homogenate, according to the established method (Matanjun et al., 2010). The absorbance of 4 preparations of blank control were averaged and used to calculate the enzyme activity. One enzyme unit is defined as the amount which inhibits 50% pyrogallol reaction.

Catalase (CAT) activity was determined on 20 μl liver and kidney homogenate diluted to 10 ml with 50 mM, pH 7.0 phosphate buffer (Matanjun et al., 2010). Two milliliters of the diluted homogenate was then mixed with 1 ml 30 mM hydrogen peroxide (H_2O_2), and the decrease in absorbance were followed for 30 s. The enzymatic decomposition of H_2O_2 in this method follows a first order reaction, and the rate constant (k) was used as a direct measure of the catalase concentration.

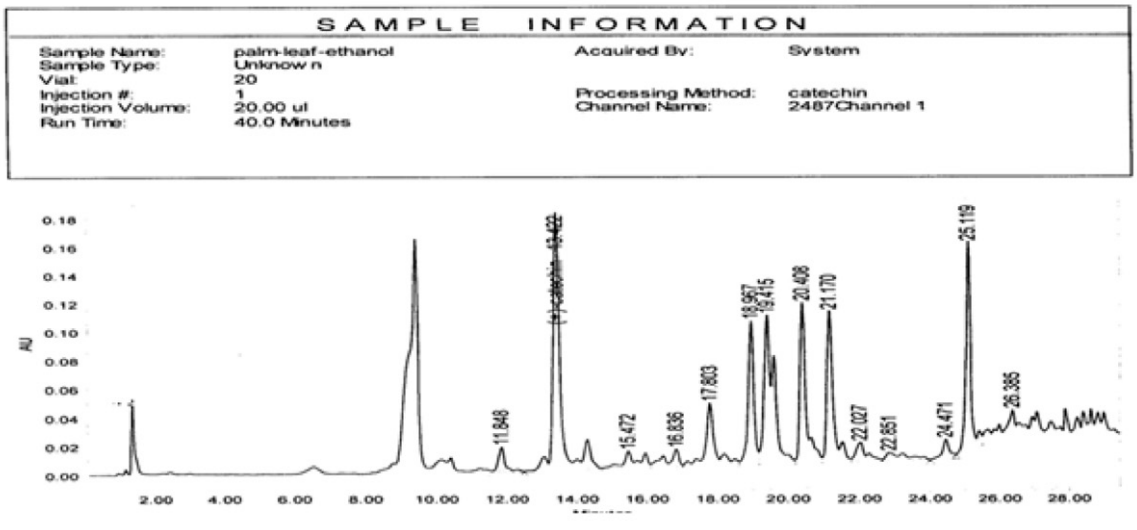
Glutathione peroxidase (GSH-Px) activities were determined on 400 μl liver and kidney homogenate (Matanjun et al., 2010). One unit of enzyme activity was defined as a decrease in the log(GSH) of 0.001 per minute after subtraction of the decrease in log(GSH) per minute for the non-enzymatic reaction.

For MDA levels, 200 μl of tissue homogenate, was diluted with 300 μl of distilled water and mixed with 35 μl of 7.0 mM BHT in ethanol, 165 μl 8.1% sodium dodecyl sulphate in distilled water, and 2 ml of thiobarbituric acid (TBA) reagent. The TBA consisted of 8 g/l TBA diluted in distilled water mixed 1:1 with 200 ml/l acetic acid and adjusted to pH 3.5 with NaOH (Matanjun et al., 2010). The mixture was immediately heated (60 min at 95 °C) and cooled with running water. One milliliter of distilled water and 4 ml of *n*-butanol were then added and mixed vigorously. The organic layer was separated by centrifugation (10 min at 3000*g*), and the absorbance of this layer was read at 532 nm against pure *n*-butanol. The method was standardised with 0–50 μM TEP, using a regression equation generated to calculate the MDA concentration in each sample. A Secomam Anthelie Advance UV–visible spectrophotometer with a 1 cm quartz cell was used for all absorbance measurements.

Minitab 13 statistical software was used for all statistical analysis. Significant differences between the groups were statistically analysed using a one-way analysis of variance (ANOVA), on the



GTEE = Green Tea leaf HPLC profile



OPLE = oil palm leaf HPLC profile; epigallocatechin EGC (0.08 %), catechin (0.30 %), epicatechin EC (0.01 %) epigallocatechin gallate EGCG (0.28%) and epicatechin gallate ECG (0.05 %)

Fig. 1. HPLC profile of green tea extract compared to oil palm leaf extract.

mean, followed by Tukey's pairwise comparison post hoc test for $p < 0.05$.

3. Results

3.1. Compounds identification of OPLE

The HPLC result of OPLE showed that the phenolic compounds in OPLE were mainly the green tea catechins, namely epigallocatechin (0.08%), catechin (0.30%), epicatechin (0.01%), epigallocatechin gallate (0.28%) and epicatechin gallate (0.05%) (Fig 1).

3.2. Histopathological assessment

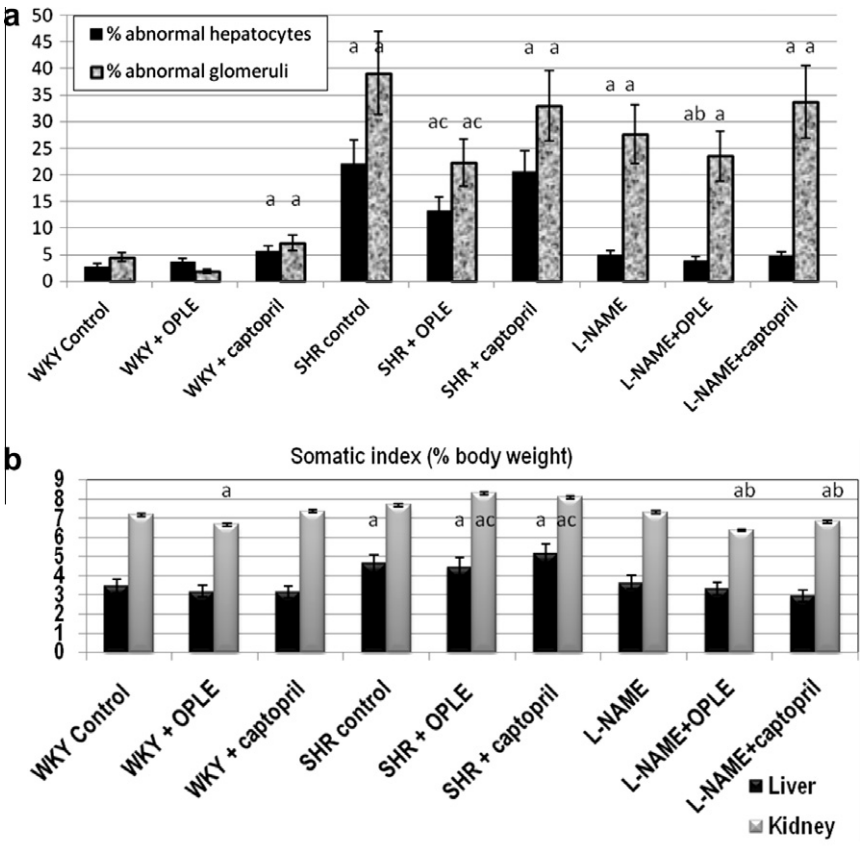
3.2.1. Liver

In SHR and NO-deficient rat livers, vacuolations and hepatocytes necrosis were evident. The SHR and NO-deficient hypertensive rat livers showed significantly higher hepatocytes injuries (22% and 4.9% respectively), compared to normal rats (2.8%) (Fig. 2). The OPLE supplementation reduced the amount of abnormal hepatocytes in SHR and NO-deficient rats to 13% and 3.8% respectively. The OPLE treatment reduced hepatocytes swelling, vacuolation and other hepatocellular injury in SHR rats, and brought the number of damaged hepatocytes to normal levels in NO-deficient rats. On the other hand, abnormal cell count increased significantly in captopril treated normotensive rats compared to the control normal rats. Hepatocytes injury has been documented as one of captopril infrequent adverse reaction. Unlike the hypertensive drugs captopril, the OPLE showed no observable injury or chronic hepatotoxicity to normal rats. Captopril treatment on SHR and NO-deficient rats showed insignificant histopathological improvement.

3.2.2. Kidneys

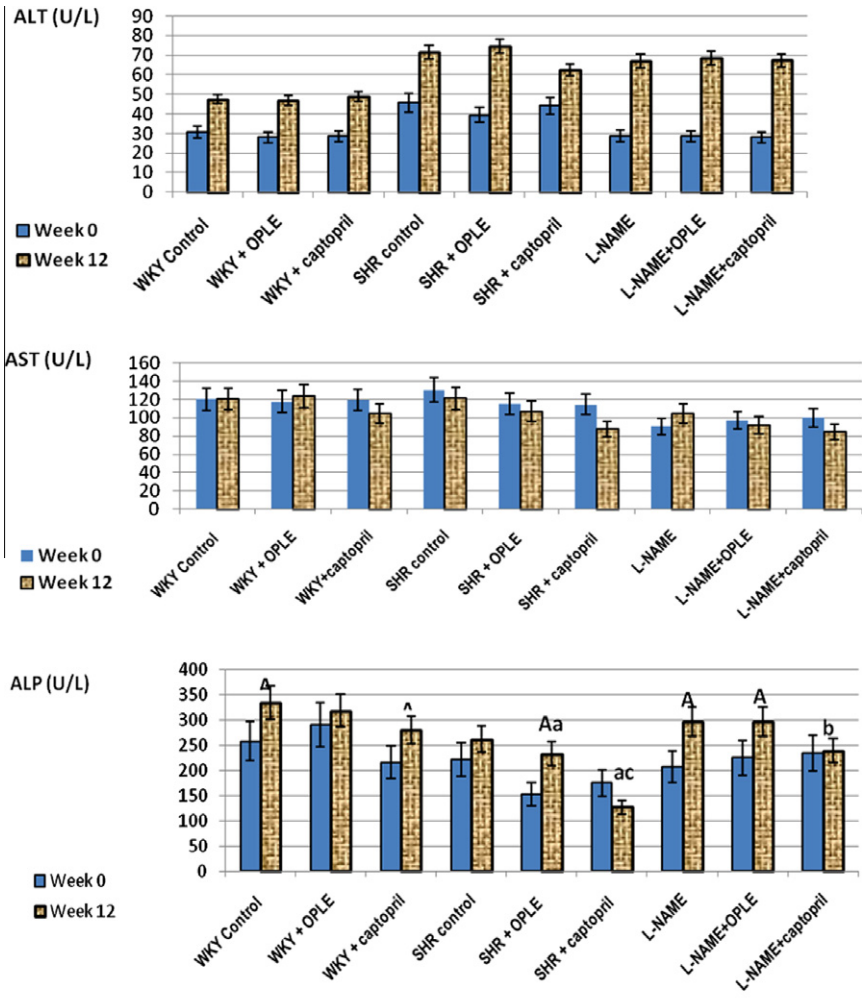
All normal rats showed minimal glomerular injury. The 3 SHR groups contained significant ($p < 0.05$) glomeruli damages (91.13% in SHR control, 87.28% in SHR + OPFME and 88.97% in SHR + captopril), compared to WKY control (Fig 2). Renal somatic indices were higher, only in the treated SHR groups, because although the kidney weights were similar, the SHR had a lower final weight. The NO-deficient rats showed marked increases in glomerular sclerotic injury ($p < 0.001$), inflammatory cells infiltrating the glomerular tuft and the surrounding area, and a small degree of glomerular adhesion to Bowman's capsule (Fig 2).

In captopril, NO-deficient and NO-deficient + captopril treated rats; the tubules were markedly swollen due to accumulation of fluid which indicated degeneration. Tubular swellings were less in NO-deficient + OPLE group but there were increases in interstitial spaces. Captopril treatment adversely affected normal rat kidneys, and many of the tubules were severely vacuolated.



WKY control = untreated normotensive Wistar Kyoto rats;
SHR control = untreated spontaneous hypertensive rats;
L-NAME: NO-deficient rats;
OPLE: Oil Palm Leaf Extract. (Mean±SD)
a $p<0.05$ vs normotensive control WKY rats; b $p<0.05$ vs NO-deficient rats.
c $p<0.05$ versus SHR control

Fig. 2. The (a) abnormal/injured hepatocytes and glomeruli (%) and (b) liver and kidney somatic indices, in SHR and NO-deficient rats compared to normal rats.



AST = aspartate aminotransferase; ALP = alkaline phosphatase; ALT = alanine aminotransferase; BUN = blood urea nitrogen. A p<0.05 versus week 0; a p<0.05 versus normotensive WKY; b p<0.05 versus untreated l-NAME; c p< 0.05 versus untreated SHR; A p<0.05 versus week 0. (mean±SD).

Fig. 3. The liver enzyme markers of SHR and NO-deficient rats compared to normal rats.

3.3. Organ damage markers

3.3.1. Liver injury biomarkers

Although the hepatocellular leakage of ALT increased with age in all rats, the net increases were highest in the SHR and NO-deficient rats (Fig. 3). Captopril significantly reduced this net ALT increases in SHR rats. Although treatments with OPLE and captopril both failed to reduce plasma ALT in NO-deficient rats, the percentage of abnormal cells in SHR + OPLE and NO-deficient + OPLE rat livers were very much lower than that of untreated SHR and NO-deficient groups. In the normotensive rats, plasma ALT of the OPLE treated rats was not raised and there was no significant change in the percentage of abnormal cells compared to the control groups. This suggests that OPLE at a daily dose of 500 mg/kg body weight, could help prevent hepatocyte damage in hypertension and NO-deficiency, and was not toxic to the normal rats' liver. Captopril reduced blood AST levels to below the normotensive WKY and untreated SHR group levels. Plasma ALP also increased with age for most untreated rat groups, and captopril treatments significantly reduced this increase, except when given to normotensive WKY rats (Fig. 3). The relative liver weights were similar in all the 6 groups. Both control and OPLE treated rat livers showed similar histological changes which were associated with ageing.

3.3.2. Kidney damage markers

The average fluid intake increased significantly in captopril ($p < 0.005$) and all NO-deficient rat ($p < 0.05$) groups (Table 1). BUN levels in NO-deficient and captopril groups were significantly higher than normal rats ($p < 0.001$) (Table 2). The NO-deficient + OPLE rats' BUN levels were similar to normal control rats and were lower than the NO-deficient control rats indicating the protective effects of OPLE. Plasma creatinine level in NO-deficient + OPLE and NO-deficient + captopril groups were significantly reduced compared to the control group ($p < 0.001$). However the levels of BUN and creatinine in all groups were in the normal ranges of 3.8–7.1 mmol/l and 44.2–97.2 μmol/l respectively (Giknis & Clifford, 2008) by the end of the study.

3.4. Oxidative stress markers

3.4.1. Kidney

The MDA levels were significantly reduced in NO-deficient + OPLE rat kidneys compared to untreated NO-deficient rats, suggesting the OPLE antioxidative properties ($p < 0.05$; Table 2). The SOD and catalase activities in most NO-deficient rat kidneys were reduced compared to control rats, although insignificantly. All groups exhibited similar GPx activity.

Table 1
Animal water intake (ml) during the experimental study.

	Average water intake (ml/rat/day)			Average food intake (g/rat/day)		
	Week 0	Week 1–6	Week 7–12	Week 0	Week 1–6	Week 7–12
WKY control	35 ± 2	33 ± 2	36 ± 2	14 ± 1	13 ± 2	16 ± 5
WKY + OPLE	38 ± 2	35 ± 3	38 ± 2	14 ± 2	14 ± 1	16 ± 2
WKY + captopril (capt)	38 ± 2	53 ± 2[d,a]	56 ± 2[d,a]	15 ± 1	15 ± 2	17 ± 5
NO-deficient control	35 ± 4	40 ± 3	41 ± 3[d,a]	14 ± 1	13 ± 2	16 ± 5
NO-deficient + OPLE	38 ± 3	49 ± 2[d,a]	45 ± 3[d,a]	14 ± 2	14 ± 1	16 ± 2
NO-deficient + capt	34 ± 2	48 ± 3[d,a]	48 ± 3[d,a]	15 ± 1	15 ± 2	17 ± 5
SHR control	34 ± 7	34 ± 3	33 ± 3	21 ± 1 [a]	24 ± 2 [a]	17 ± 2
SHR + OPLE	32 ± 3	32 ± 6	33 ± 6	18 ± 3	20 ± 2 [a]	20 ± 4 [a]
SHR + captopril	40 ± 9	48 ± 2[a]	39 ± 4	20 ± 4 [a]	25 ± 2 [a]	20 ± 2 [a]

Results are expressed as mean ± SEM. WKY control, Wistar Kyoto rats without any treatment; WKY + OPLE, WKY treated with oil palm leaves alcoholic extract; WKY + captopril, WKY treated with captopril; WKY + L-NAME, WKY treated with L-NAME; L-NAME + OPLE, WKY treated with L-NAME + OPLE; L-NAME + captopril, WKY treated with L-NAME + captopril; SHR control, spontaneously hypertensive rats without any treatment; SHR + OPLE, SHR treated with oil palm leaves alcoholic extract; SHR + captopril, SHR treated with captopril.

[a] Significantly different ($p < 0.05$) compared to WKY control.
[b] Significantly different ($p < 0.05$) compared to WKY + L-NAME (NO deficient).
[c] Significantly different ($p < 0.05$) compared to SHR control.
[d] Significantly different ($p < 0.05$) compared to week 0.

Table 2
Rats biophysical and biochemical kidney function tests, histopathology, and antioxidant status during the experimental study.

Groups	Plasma BUN (mmol/L)		Plasma creatinine (μmol/L)	
	Week 0	Week 12	Week 0	Week 12
WKY control	6.55 ± 0.25	5.83 ± 0.26	53.8 ± 2.2	70.3 ± 0.9
WKY + OPLE	7.64 ± 0.18	5.88 ± 0.30	55.8 ± 1.4	71.8 ± 2.0
WKY + Captopril	6.64 ± 0.44	10.40 ± 0.97[a]	53.9 ± 1.4	69.3 ± 1.3
NO-deficient control	6.17 ± 0.37	10.10 ± 1.03[a]	61.0 ± 2.9	68.3 ± 6.0
NO-deficient + OPLE	6.42 ± 0.32	6.96 ± 0.66[b]	53.0 ± 2.3	60.0 ± 2.2[a]
NO-deficient + captopril	7.31 ± 0.74	8.27 ± 0.40[a]	53.3 ± 0.9	61.4 ± 1.4[a]
SHR control	7.54 ± 0.09[a]	7.13 ± 0.11[a]	62.0 ± 1.6[a]	63.3 ± 1.4[a]
SHR + OPLE	6.70 ± 0.18	7.13 ± 0.17[a]	60.0 ± 0.1	53.7 ± 0.9[a,c]
SHR + captopril	7.13 ± 0.20	7.08 ± 0.13[a]	67.0 ± 1.4[a]	52.8 ± 1.1[a,c]
Groups	MDA (nmol/g tissue)	Final body weight (g)	Kidney weight (g)	
WKY control	36.58 ± 0.09	333.4 ± 6.7	2.39 ± 0.13	
WKY + OPLE	36.52 ± 0.18	325.0 ± 11.1	2.15 ± 0.07	
WKY + Captopril	41.94 ± 1.28	291.4 ± 8.6	2.14 ± 0.07	
NO-deficient control	52.76 ± 0.73[a]	333.8 ± 13.8	2.33 ± 0.11	
NO-deficient + OPLE	36.38 ± 0.66[b]	311.2 ± 23.7	1.96 ± 0.15	
NO-deficient + captopril	37.04 ± 1.20[b]	296.4 ± 6.0	2.017 ± 0.07	
SHR control	37.44 ± 1.49	264.9 ± 6.2[a]	2.05 ± 0.07	
SHR + OPLE	30.58 ± 1.04	270.3 ± 8.4[a]	2.26 ± 0.07	
SHR + captopril	40.54 ± 0.96	259.1 ± 6.2[a]	2.07 ± 0.08	
	Kidney tissues Antioxidant enzymes /mg protein			
	SOD (unit)	Catalase (k)	GPx (unit)	
WKY control	2.60 ± 0.11	26.9 ± 2.8	2.42 ± 0.10	
WKY + OPLE	2.50 ± 0.07	33.2 ± 1.2	2.58 ± 0.22	
WKY + Captopril	2.45 ± 0.14	34.8 ± 1.3	2.41 ± 0.10	
NO-deficient control	1.87 ± 0.22	18.4 ± 1.8	2.73 ± 0.54	
NO-deficient + OPLE	1.84 ± 0.19	18.7 ± 1.4	2.58 ± 0.16	
NO-deficient + captopril	2.24 ± 0.63	20.9 ± 3.1	2.30 ± 0.21	
SHR control	2.34 ± 0.07	29.4 ± 1.1	2.88 ± 0.10	
SHR + OPLE	0.67 ± 0.09[a,c]	25.8 ± 1.0	1.72 ± 0.09[a,c]	
SHR + captopril	0.80 ± 0.10[a,c]	26.0 ± 1.9	2.02 ± 0.09[a,c]	

Results are expressed as mean ± SEM. WKY control, Wistar Kyoto rats without any treatment; WKY + OPLE, WKY treated with oil palm leaves alcoholic extract; WKY + captopril, WKY treated with captopril; WKY + L-NAME, WKY treated with L-NAME; L-NAME + OPLE, WKY treated with L-NAME + OPLE; L-NAME + captopril, WKY treated with L-NAME + captopril; SHR control, spontaneously hypertensive rats without any treatment; SHR + OPLE, SHR treated with oil palm leaves alcoholic extract; SHR + captopril, SHR treated with captopril.

[a] Significantly different ($p < 0.05$) compared to WKY control.
[b] Significantly different ($p < 0.05$) compared to NO-deficient control.
[c] Significantly different ($p < 0.05$) compared to SHR control.

3.4.2. Liver

In the SHR and NO-deficient rat livers, the MDA levels, were not significantly different from that of the normotensive control group (Table 3). The OPLE supplementation caused an insignificant lowering of the MDA levels in WKY control and NO-deficient rat livers. The OPLE treatment significantly decreased SOD activities in SHR and NO-deficient rat livers compared to their respective untreated controls.

4. Discussion

4.1. Liver

The hepatic microcirculation is unique because 90% of blood flow enters through the portal vein, and resistance changes occur in the hepatic sinusoid microvascular network (McCuskey & Reilly, 1993). Under hypertension and NO-deficiency, disruption of liver

Table 3
Activities of SOD, catalase and GPx, MDA level in the rat livers.

Experimental Group	MDA (nmol/g tissue)	SOD (unit/g protein)	GPx (unit/g protein)	Catalase (unit/g protein)	Systolic B.P at 12 weeks
Control	29.5 ± 4.2	2.60 ± 0.06	1.76 ± 0.31	46.2 ± 3.4	110 ± 5
OPLE	25.4 ± 5.8	2.37 ± 0.31	2.09 ± 0.13	60.0 ± 3.6	108 ± 5
Captopril	21.6 ± 4.3	2.78 ± 0.26	2.49 ± 0.26	44.6 ± 10.4	95 ± 10
SHR control	24.2 ± 4.3	2.33 ± 0.20	2.42 ± 0.21	37.8 ± 1.2	190 ± 10[a]
SHR + OPLE	24.5 ± 4.3	0.95 ± 0.13[a,c]	0.73 ± 0.06[c]	30.1 ± 3.7[a]	190 ± 11[a]
SHR + captopril	24.0 ± 4.3	1.02 ± 0.10[a,c]	1.61 ± 0.18	36.8 ± 2.8	140 ± 5[a]
NO-deficient control	30.5 ± 4.8	2.14 ± 0.05[a]	1.91 ± 0.07	45.1 ± 6.2	180 ± 10[a]
NO-deficient + OPLE	26.3 ± 4.9	1.75 ± 0.06[a,b]	1.92 ± 0.369	38.6 ± 4.9	110 ± 5[b]
NO-deficient + captopril	40.8 ± 8.0	2.35 ± 0.12	2.22 ± 0.199	36.6 ± 1.6	95 ± 10[b]

Results are expressed as mean ± SEM. [x]Hepatosomatic index were similar in all groups; WKY control, Wistar Kyoto rats without any treatment; WKY + OPLE, WKY treated with oil palm leaves alcoholic extract; WKY + captopril, WKY treated with captopril; WKY + L-NAME, WKY treated with L-NAME; L-NAME + OPLE, WKY treated with L-NAME + OPLE; L-NAME + captopril, WKY treated with L-NAME + captopril; SHR control, spontaneously hypertensive rats without any treatment; SHR + OPLE, SHR treated with oil palm leaves alcoholic extract; SHR + captopril, SHR treated with captopril.

[a] Significantly different ($p < 0.05$) compared to WKY control.

[b] Significantly different ($p < 0.05$) compared to NO-deficient control.

[c] Significantly different ($p < 0.05$) compared to SHR control.

tissue architecture and vacuolation, is an indication of hepatic fatty infiltration and hepatocellular injury (Hoetzel et al., 2008). The NO is important in maintaining hepatic microvasculature and local NO-deficiency may contribute to local microvascular perfusion failure, portal hypertension, hepatic resistance increase, and mimic other vascular dysfunctions like arteriosclerosis. One mode, by which OPLE helped protect the hepatocytes from severe injury under NO-deficiency, is through their BP lowering effects. The OPLE may have increased endothelium dependent hyperpolarizing factor (EDHF) and reduced the portal pressure as indicated by the reduced BP of OPLE supplemented NO-deficient rats. The endothelium regulates vasomotor tone, vascular homeostasis, and inflammatory processes through the production of various substances in response to diverse physical and chemical stimuli (Behrendt & Ganz, 2002). A decreased vascular NO level in endothelial cells is indicative of endothelial dysfunction. Although lower BP would improve hepatic microvascular perfusion and indirectly preserve the hepatocytes from damage, captopril which effectively normalised the BP, did not protect against damages caused by the NO-deficiency.

In SHR rats, the BP was not reduced by the OPLE supplementation, yet the hepatoprotective effects were very obvious. Thus normalising the BP is not the sole mechanism for the hepatocellular protection by OPLE against the extensive injury caused by essential hypertension or NO-deficiency. Hepatic injury are linked to dyslipidemia, low-grade inflammation and serum NO linked insulin resistance. Green tea catechins protective mechanisms against tissue damage included through (i) antioxidant actions, (ii) central nervous system effects, (iii) gut transport alterations, (iv) fatty acid sequestration and processing, (v) peroxisome proliferator-activated receptor (PPAR) activation, (vi) increasing insulin sensitivity (Prasain, Carlson, & Wyss, 2010), (vii) inflammatory and endothelial apoptosis modulation (Curin & Andriantsitohaina, 2005) (viii) enhancing vascular NO production (Galleano, Oteiza and Fraga, 2009), (ix) maintaining endothelial functions and vascular homeostasis (Moore, Jackson, & Minihane, 2009). EGCG helped prevent hypertrophy and hypertension by suppressing (i) Ang II- and pressure-overload (ii) reactive oxygen species (ROS) generation and NADPH oxidase over expressions, (iii) NF-kappaB and AP-1 activation, (iv) ROS-dependent p38 and c-Jun N-terminal kinase (JNK) signalling pathways, (v) EGFR transactivation (vi) extracellular signal-regulated kinase (ERKs)/PI3 K/Akt/mTOR/p70(S6 K) and (vii) reactivation of ANP and BNP (Atrial and Brain natriuretic peptide, markers of chronic heart failure), and involved various intracellular signalling transductional pathways inhibition (Li et al., 2006). Epicatechin, its procyanidins oligomers, and especially the tetramer reportedly inhibited acetylcholine esterase (ACE) activity (Ottaviani, Actis-Goretta, Villordo, & Fraga, 2006). EGCG but not EGC, inhibited Ang II-stimulated (i) vascular smooth muscle cell (VSMC) hypertrophy, (ii) JNK signalling pathway at transcriptional and posttranslational levels, but apparently not through ERK and p38 Mitogen-activated protein kinases (MAPK) (Zheng et al., 2004). Green tea catechins were reported to protect brain, liver, and kidney from lipid peroxidation injury (Sano, Takahashi, Yoshino, Tomita, Oguni, & Konomoto, 1995). The OPLE reportedly caused vasorelaxation in rat artery through an endothelium dependent and independent mechanisms (Abeywardena, et al., 2002), and was associated to its high phenolic contents (Runnie et al., 2003).

The present results suggest an *in vivo* organ antioxidant effect of OPLE, as the down-regulation of SOD could be due to a decreased level of oxidative stress, stimulating a decreased need in the first line of endogenous antioxidant defence. This is demonstrated even more in SHR rats where OPLE supplementation caused all the antioxidant enzymes (SOD, GPx and catalase) to be significantly down-regulated without causing significant increases in MDA levels. It indicated that with the OPLE supplementation, less antioxidant enzymes activities were required to keep the MDA level at a normal physiological value. Enhanced lipid peroxidation which is associated with antioxidant depletion in different tissues, may yield a range of toxic aldehydes that are capable of damaging membrane proteins (Husain, Scott, Reddy, & Somani, 2001), leading to the leakage of cytoplasmic enzymes (Bagchi, Bagchi, Adickes, & Stohs, 1995). The reduction in portal pressure may be linked to the increased bioactivity of NO in the sinusoidal endothelial cells, since the highly reactive ROS, can reduce the half-life of NO.

ALT is found mainly in the liver, and much less in the kidneys, heart, or skeletal muscle. Elevation of ALT suggests, but does not confirm, the existence of problems such as hepatitis, liver damage, bile duct problems, congestive heart failure, infectious mononucleosis, and myopathy. Fluctuation of ALT levels may occur during the day, and can increase after a strenuous physical exercise. Some drugs can also elevate ALT levels (Giboney, 2005). ALT is a more specific indicator of liver inflammation than AST, as AST may increase in other organ diseases. AST is linked to liver parenchymal cells and is raised in acute liver damage, but is also present in red blood cells and cardiac muscle, skeletal muscle, kidney and brain tissue, and may be raised when these tissues are injured. The AST/ALT ratio is useful in differentiating between hepatotoxicity caused by chemical (>2.0) or virus (<1.0), but is not relevant when the liver enzymes are not elevated, or where multiple conditions co-exist. Elevation of ALT due to liver-cell damage can be distinguished from biliary duct problems by measuring alkaline

phosphatase. ALP occurs in many tissues, but is concentrated in the liver, bile duct, kidney, bone and placenta. High ALP levels occur when the bile ducts are blocked. ALP is also higher in children, pregnant women and when the osteoblast is active (Coleman, 1992).

4.2. Kidney

Hypertension is major risk factor that predisposes the kidney to disorders, which is responsible for morbidity and mortality in patients. Microvascular changes alters kidney perfusion and causes hypertension-related damages. Pharmacological agents used to lower BP, can help prevent/reduce hypertension-related kidney damage. Polyphenols, with its BP lowering activity, have been shown to protect from these damages (Bernátová et al., 2002).

Inadequate NO production causes renal vasoconstriction and BP increase, which consequently mediates the complex hemodynamic disorders associated with chronic renal disease (Schmidt & Baylis, 2000). However, intra-renal local and low-dose systemic NOS inhibition can generate renal vasoconstriction without BP increase. Partial NO blockade over an extended period can produce a moderate, stable hypertension with renal vasoconstriction, proteinuria and mild glomerular sclerotic injury. The L-NAME caused an increase in water intake with or without elevation in systemic arterial pressure. Similarly, L-NAME also caused an increase in water excretion, associated with the obstruction of antidiuretic hormone release from the pituitary due to the important neural activity of NO.

There should more than 70% of non-functional nephrons before any significant elevations in plasma BUN or creatinine levels can be observed. Here, the glomerular damages caused by NO-deficiency were less than 35% and renal atrophy was absent. However a certain degree of renal insufficiency could be present during NO-deficiency as significantly elevated BUN level was observed, and treatment with OPLE successfully reduced BUN level to normal. This reflects the protective effect of OPLE on glomerular function, as the presence of abnormal glomerulus was slightly lower compared to NO-deficient group. The dose of L-NAME used in this study is relatively low compared to other studies (Miao, Shen, & Su, 2001) to allow for a longer duration of work, hence the comparatively mild glomerular injury. In this study, the NO-deficient rats SBP were benign and maintained moderately between 160 and 180 mm Hg, and there was no sudden accelerated increment.

The renal MDA levels in NO-deficient rats were dramatically increased, and both OPLE and captopril treatments diminished these increases, again proving their antioxidant properties. Potent antioxidants generally retard lipid peroxidation, and the OPLE afforded protection against the L-NAME mediated lipid peroxidation enhancement, characteristic of this model (Bapat et al., 2002). The reduction of oxidative stress in the kidney by OPLE or captopril preserves the bioavailability of residual NO and hence reduces BP.

Sodium excretion occurs in the tubules and any fall in the renal interstitial fluid pressure will affect sodium and water excretion, causing fluid accumulation in the tubular cells. Excess protein infiltration, which is common in hypertension, also generates tubular damage, inflammation and scarring. Blockade of NO decreases renal perfusion, resulting in decreased papillary blood flow and impaired sodium excretion. Both OPLE and captopril failed to attenuate tubular damage during NO-deficiency as evidenced by the presence of dilated tubules and increased peritubular space. This suggested impairment of Na^+ transport and accumulation of water due to osmotic gradient, although it was less extensive with the OPLE treatment, as indicated by the normal BUN level. The tubular structural abnormalities lead to impairment of urine concentrating ability and caused diuresis and increased water consumption, as observed in all NO-deficient rats.

In the normal rats with OPLE treatment, a few scattered patches of mildly dilated tubular cells was present, but it was not accompanied by elevated BUN, creatinine and MDA levels, which indicated that the overall renal function was not impaired. Lower doses of OPLE (300 mg OPLE/kg body weight per day, results not presented here) produced normal tubular cells. Tubular damage is commonly seen with drugs which induce nephrotoxicity such as gentamicin and cisplatin though the extent of tubular injury in these drugs is usually quite severe. This unwanted effect is uncommon but has been reported in a human case of *Cupressus funebris* ingestion which is rich in flavonoids (Lee & Chen, 2006). Captopril treated rats also exhibited tubular changes to a greater extent than the OPLE treated group.

Essential hypertension is frequently associated with liver and renal damages, and depends on the degree to which the microcirculation is exposed to the elevated blood pressure. Oxidative stress and alteration of cellular redox state, are linked to many types of acute and chronic liver and kidney injury (Dey & Cederbaum, 2006). Lipid peroxides could alter biological membranes properties, resulting in severe cell damage. This study provides evidence of the protective effects of OPLE in 2 important hypertensive target organs under normal and NO-deficient environment. The extent of organ protection was not complete but the injury observed was considerably attenuated. The kidney plays an important role in regulating BP and at the same time longstanding high BP causes vascular changes that adversely affect the organ function.

The OPLE significantly ($p < 0.05$) attenuated BP increases, increased serum NO, reduced lipid peroxidation and showed antioxidant effects in NO-deficient hypertensive rats.

5. Conclusion

This study showed that catechin-rich OPLE on its own at 500 mg/kg body weight/day was not toxic to normal rats. The OPLE help protect the liver and kidney from potential damage caused by hypertension and NO-deficiency. This is the first report on the *in vivo* liver and kidney protective properties of green tea catechins extracted from an alternative source namely oil palm leaf for use as functional food.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.foodchem.2011.03.050.

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