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Citrus leaf extract reduces blood pressure and vascular damage in repeatedly heated palm oil diet-Induced hypertensive rats

Hawa Nordin Siti[a,b], Yusof Kamisah[a], Mohd Ishak Nur Iliyani[c], Suhaila Mohamed[c], Kamsiah Jaarin[d,*]

[a] Department of Pharmacology, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, Kuala Lumpur, Malaysia
[b] Department of Basic Medical Sciences, Faculty of Medicine, Universiti Sultan Zainal Abidin, Terengganu, Malaysia
[c] Institute of Bioscience, Faculty of Food Science and Technology, Universiti Putra Malaysia, Selangor, Malaysia
[d] Department of Pharmacology, Faculty of Medicine and Defence Health, National Defence University of Malaysia, Kem Sungai Besi, Kuala Lumpur 57000, Malaysia

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ABSTRACT

Prolonged consumption of repeatedly heated vegetable oil increases blood pressure. This study aimed to determine the effects of Citrus leaf extract, (CLE) on blood pressure, blood pressure-regulating enzymes and mediators, as well as aortic histomorphometry in heated palm oil induced-hypertension. Male *Sprague Dawley* rats ($n = 56$) were divided into seven groups; control group was given normal diet and the other groups were fed with palm oil-enriched diet (15% w/w) either fresh (FPO), five-time-heated (5HPO) or ten-time-heated (10HPO) with or without CLE (0.15%, w/w) supplementation. CLE supplementation reduced the heated oil-raising effect of blood pressure, plasma TBARS, thromboxane and angiotensin-1 converting enzyme in 5HPO but not in 10HPO group. CLE increased serum heme oxygenase-1 in both 5HPO and 10HPO groups. CLE supplementation reduced the increase in aortic intima-media thickness, intima-media area and circumferential wall tension in 5HPO group but not in 10HPO group. These findings suggested that CLE supplementation reduces the blood pressure-raising effects of 5HPO and vascular damage, possibly through its antioxidant effect by modulating vasoactive mediators and blood pressure-regulating enzymes.

1. Introduction

Fresh vegetable oils, which are rich in unsaturated fatty acids and antioxidants, are beneficial in reducing risk of cardiovascular disease [1]. The oils are commonly used for frying in food preparation and repeatedly used to save cost [2]. This habit is practiced not only in developing countries but also in developed countries [3,4]. However, the quality of the oils deteriorates after repeated heating, which includes increased viscosity and darkening in color, due to altered fatty acid composition of the oil [5].

Heated vegetable oil undergoes thermal oxidation and a series of chemical reactions which generate polar compounds and reactive oxygen species (ROS), namely hydroxyl, alkoxy and peroxyl radicals, leading to increased oxidative stress [6]. Oxidative stress promotes vascular inflammation, endothelial dysfunction, and imbalance between vasodilation and vasoconstriction agents which eventually lead to the development of hypertension [7–13]. Previous studies in rats showed that prolonged intake of repeatedly heated palm and soy oil increased lipid peroxidation [12,13] and blood pressure [8–12] as well as causing the imbalance between vasodilator and vasoconstrictor mediators in favor of vasoconstriction [11,15]. Furthermore, studies by Ng et al. [9] reported the involvement of adverse vascular remodeling in the heated vegetable oil-induced hypertension in rat model.

The extract (CLE) is derived from Citrus spp. leaves (*Rutaceae* family) consisting of combinations of *C. hystrix, C. aurantifolia, C. microcarpa* and *C. sinensis* [16–18], developed and patented (patent number: US8425969B2) by Universiti Putra Malaysia researchers. It is used to reduce vegetable oil thermal oxidation. [19]. The established extraction methods for acquiring CLE was designed to effectively isolate and extract polyphenols in particular the flavonoids, such as diosmin, lutein, obacunone, isoquercitrin, hesperidin, didymin, eriocitrin, neocriocitrin, narirutin, naringin, neohesperidin and 7-OH flavonone [19]. Flavonoids are the most abundant polyphenols subgroup detected in citrus plants in Malaysia [16–18]. Previously, Sukalingam et al. [20] shown that

* Corresponding author.
E-mail address: kamsiahjaarin@gmail.com (K. Jaarin).

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CLE which was also named as ADD-X (patent no: US 8425969B2), reduced blood pressure and improved oxidative stress status in atherosclerotic rat model. Citrus species generally have antihypertensive effect attributed to their flavonoid-rich composition except for *citrus aurantium* [21–25]. We postulated that CLE may prevent blood pressure-raising effect of heated oil by regulating the vasoactive mediators and vascular remodeling apart from antioxidant effect. Therefore, this study was undertaken to evaluate the effects of CLE extract on blood pressure, blood pressure-regulating enzymes and mediators including angiotensin converting enzyme (ACE), heme oxygenase-1 (HO-1), thromboxane (TXA_2) and prostacyclin (PGI_2), lipid peroxidation (TBARS) as well as vascular histomorphometry in rats fed with heated oil.

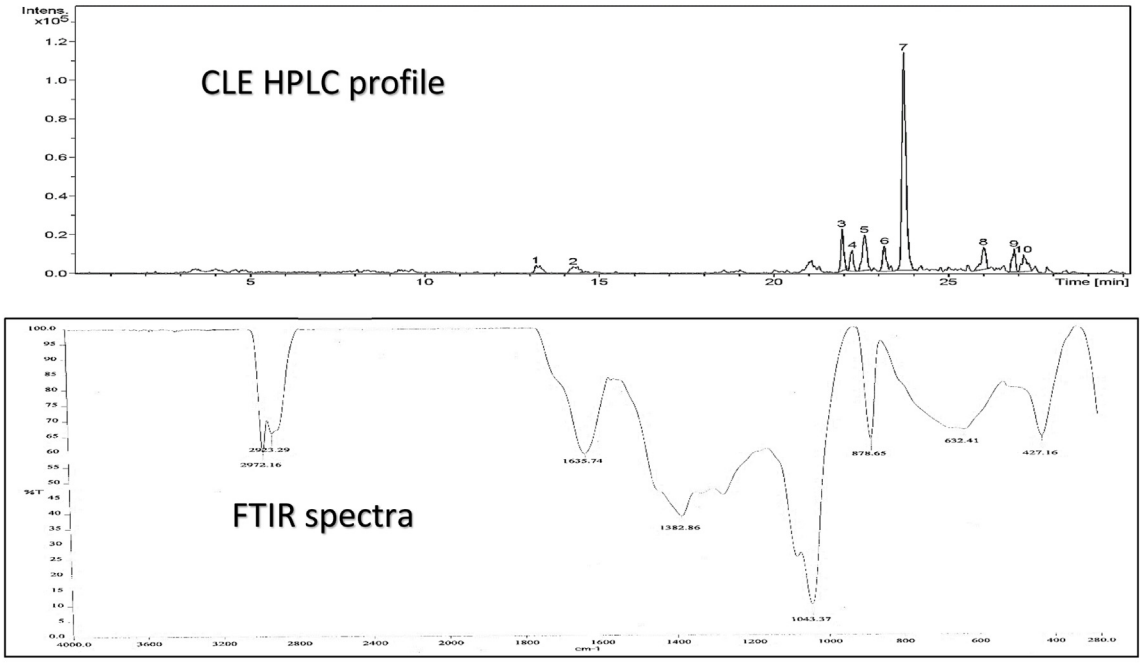
2. Materials and methods

2.1. Materials

CLE was obtained from Institute of Bioscience, Universiti Putra Malaysia, Selangor. Palm oil was purchased from Lam Soon Edible Oils, Selangor, Malaysia. TBARS, thromboxane B_2 and 6-keto-$PGF_{1\alpha}$ kits were purchased from Cayman Chemical, Ann Arbor, MI, USA.

LC-MS elucidation of polyphenols in *CLE*.

Peak	T_R (min)	Precursor ion $[M+H]^+$	Formula	Fragment ions (m/z)	Identification
1	13.19	609.16	$C_{28}H_{32}O_{15}$	203, 305, 609	Diosmin
2	14.24	567.31	$C_{40}H_{56}O_2$	567	Lutein
3	21.94	500.38			Unknown
4	22.22	456.36			Unknown
5	22.57	456.36	$C_{26}H_{30}O_7$	456, 618	Obacunone
6	23.14	468.39	$C_{21}H_{20}O_{12}$	468	Isoquercitrin
7	23.69	424.37			Unknown
8	25.97	593.23	$C_{28}H_{34}O_{14}$	593, 809	Didymin
9	26.85	607.24	$C_{28}H_{34}O_{15}$	607	Hesperidin
10	27.12	621.26			Unknown



FTIR DATA OF CLE (CITRUS spp. LEAF EXTRACT)

Sample	Frequency (cm^{-1})				
	O-H (carboxylic acids)	C=C (alkene)	-C-H (bending)	C-O stretching vibrations (ester)	=C-H (bending)
CLE	2972 and 2923	1635	1382	1043	878

Fig. 1. Chemical characteristics of CLE determined by HPLC, LC–MS and FTIR. HPLC, high performance liquid chromatography; LC–MS, liquid chromatography–mass spectrometry; FTIR, fourier transform infrared spectroscopy.