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Protection of Chinese olive fruit extract and its fractions against advanced glycation endproduct-induced oxidative stress and pro-inflammatory factors in cultured vascular endothelial and human monocytic cells ☆

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ABSTRACT

Accumulation of advanced glycation endproducts (AGEs) plays a pivotal role in the pathophysiology of diabetic complications. In this study, we evaluated the protective activities of water/ethanol (1:1, v/v) extract (WEE) and its fractions from Chinese olive (*Canarium album* L.) fruit on AGEs-induced endothelial dysfunction. The WEE and its fractions could scavenge free radicals, inhibit AGEs formation and reduce the overgeneration of AGEs-induced ROS in endothelial cells. They also increased AGEs-mediated loss of glutathione level and SOD activity. AGEs stimulated monocyte adhesion, ICAM-1 expression and IκBα phosphorylation, which were inhibited by WEE and its fractions. Furthermore, they reduced AGEs-induced inflammatory cytokines including TNFα, interleukin-1β and interleukin-6 level in monocytes. Nine compounds such as gallic acid, methyl gallate, ethyl gallate, scopoletin, ferulic acid, ellagic acid, and rutin were identified. These findings indicated that Chinese olive fruit attenuated AGEs-mediated endothelial dysfunction by ameliorating inflammation and oxidant stress responses via inhibiting NF-κB pathway.

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1. Introduction

Diabetes mellitus (DM), a group of metabolic diseases characterised by abnormal glucose metabolism, is a serious and growing health problem worldwide and is associated with severe acute and chronic complications that negatively influence both the quality of life and survival of affected individuals. The global figure of the number of adults with diabetes was estimated at 382 million in 2013 and is expected to rise to 592 million in 2035 (Guariguata et al., 2014). Chronic hyperglycaemia may contribute to the pathogenesis of long-term DM complications and has an important role in increasing morbidity and mortality (Marcovecchio, Lucantoni, & Chiarelli, 2011). Glycation, the non-enzymatic reaction of reducing sugars with amino groups, is increased in hyperglycaemic physiological environments, leading to an acceleration of the formation of advanced glycation endproducts (AGEs). The oxidative steps are also involved in glycation and the process can therefore be also called glycoxidation. Increased accumulation of AGEs and oxidative stress can induce multiple cellular changes leading to macro- and microvascular complications, such as atherosclerosis, diabetic retinopathy, nephropathy, and neuropathy (Ahmed, 2005; Goldin, Beckman, Schmidt, & Creager, 2006).

Cardiovascular diseases, including accelerated atherosclerosis and microangiopathy, are typical diabetic complications related to an early endothelial dysfunction process (Schalkwijk & Stehouwer, 2005). The mechanisms of diabetic cardiovascular diseases are multifaceted, involving increased oxidative/nitrosative stress, accumulation of AGEs, enhanced receptor for advanced glycation end product (RAGE), activation of various pro-inflammatory and cell death signalling pathways, and increased adhesion of circulating monocytes to the vessel wall, etc. (Ramasamy, Yan, & Schmidt, 2011). AGEs exert their damaging effects by binding to RAGE on the surfaces of various cells such as endothelial cells, macrophages, and smooth-muscle cells. Binding of AGE to RAGE causes oxidative stress and activates large amounts of pro-inflammatory cytokines, including interleukin-1 (IL-1) family, and tumour necrosis factor α (TNFα). These pro-inflammatory cytokines activate nuclear factor-κB (NF-κB) and mitogen activated protein kinase (MAPK) signalling pathways. These molecules in turn induce the expression of adhesion molecules on the cell surface such as vascular cell adhesion molecule-1 (VCAM-1) and intracellular adhesion molecule-1 (ICAM-1), which are centrally involved in the progression of atherosclerotic lesions. These have been suggested to be the key player in the generation of both micro- and macrovascular diabetes complications (Prangthip et al., 2013; Yamagishi, 2011).

AGEs mediated inflammation and endothelial dysfunction have been known as a key underlying cause in the development of vascular complications (van den Oever, Raterman, Nurmohamed, & Simsek, 2010). Therefore, many efforts have been extended to search dietary plants and fruits which effectively inhibit AGEs formation. It has been reported that antioxidants and radical scavengers inhibit the glycation processes (Deetae, Parichanon, Trakunleewatthana, Chanseetis, & Lertsiri, 2012; Wu, Hsieh, Wang, & Chen, 2009b) and may play a theoretical strategy for preventing diabetic complications (Matough, Budin, Hamid, Alwahaibi, & Mohamed, 2012). In addition, recent studies have shown that compounds with combined antioxidant and antiglycation properties are more effective in treating diabetes mellitus (Soman, Rauf, Indira, & Rajamanickam, 2010). Therefore, investigations on AGEs inhibitors could present a potential preventive and therapeutic method for lowering the development of diabetic complications. Due to the side effects of the existing synthetic drugs, plant derived functional foods are in great demand in developing countries as an alternative approach to treat diabetes.

Chinese olive (*Canarium album* L.) is widely cultivated in Taiwan and other Asian regions. The fresh fruit of Chinese olive are generally processed in the food industry to beverages, candy and confections. Chinese olive fruit is used in folk medicine to relieve sore throats, quench thirst, combat diarrhoea, and promote the production of body fluid and detoxicating. Some pharmacological functions such as hepatoprotective (Ito et al., 1990), antimicrobial (Yuan, Liu, & Tang, 2001) and antivirus (Kong et al., 1998) properties of Chinese olive fruits have been demonstrated. Mogana and Wiart (2011) have reviewed the potential pharmacologic activities and phytochemical of the extract from the fruit, leaf or bark of *Canarium* spp. In spite of the pharmacological potentials, Chinese olive fruit is still very much under studied. Moreover, little is known regarding the antidiabetic activity of the Chinese olive fruit. Previous studies in our lab revealed that the water/ethanol (1/1, v/v) extract (WEE) from Chinese olive fruits exhibited well scavenging effects on free radicals, antioxidant activity towards lipid and protein, and inhibitory effects on AGEs formation. Phytochemical analysis had shown that Chinese olive fruit was rich in phenolic compounds and triterpenoids. Gallic acid, ferulic acid and rutin were isolated from WEE (Kuo, Liu, Hsu, Lin, & Chen, 2015). However, the mechanism of protection of Chinese olive extract against AGEs-induced injury was not elucidated well and reports were scanty. Therefore, in this study, we investigated the beneficial effects of Chinese olive fruit extract and its fractions on AGEs-induced inflammation and monocyte-endothelial dysfunction.

2. Materials and methods

2.1. *Chemicals*

2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), glucose, catechin, bovine serum albumin (BSA), 1,1-diphenyl-2-picrylhydrazyl (DPPH), sodium azide, 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), L-cysteine, aluminium chloride hexahydrate ($AlCl_3 \cdot 6H2O$) 2′,7′-dichlorodihydrofluorescein diacetate (H_2DCFDA), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), linoleic acid and all HPLC standards were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Ammonium thiocyanate and Folin–Ciocalteau reagent were purchased from E. Merck Co. (Darmstadt, Germany). Foetal bovine serum (FBS), trypsin-EDTA, penicillin-streptomycin (PSN), RPMI-1640, Dulbecco's modified eagle medium (DMEM) and Dulbecco's phosphate-buffered saline (PBS), ELISA kits for the detection of ICAM-1, IL-1β, IL-6 and TNFα were obtained from Invitrogen (Frederick, MD, USA). ELISA kits for the detection of phosphorylated and total IκBα were purchased from eBioscience (San Diego, CA, USA). Kits for the detection of glutathione (GSH)