3,4-DI-*O*-CAFFEOYL QUINIC ACID PROTECTS ENDOTHELIAL CELLS AGAINST OXIDATIVE STRESS AND RESTORES ENDOTHELIUM-DEPENDENT VASODILATATION

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We investigated the cytoprotective effect of 3,4-di-*O*-caffeoyl quinic acid, a phenolic compound isolated from *Elephantopus mollis* H. B. K. (Compositae), on oxidative stress-induced cell damage in vascular endothelial cells. Result showed that 3,4-di-*O*-caffeoyl quinic acid (1 to 30 μM) displayed potent DPPH radical scavenger activity more efficient than resveratrol. Free radical-mediated oxidative modification of cell membrane by Fenton's reagent ($H_2O_2/FeSO_4$) was also significantly attenuated by 3,4-di-*O*-caffeoyl quinic acid as measured by thiobarbituric acid-reactive substances (TBARS). Furthermore, this compound not only effectively minimized the loss of cell viability induced by Fenton's reagent in cultured human umbilical vein endothelial cells (HUVEC) but also significantly reversed $H_2O_2/FeSO_4$-induced impairment of endothelium-dependent relaxation to acetylcholine in rat aorta. These data suggested that 3,4-di-O-caffeoyl quinic acid prevents cell from oxidative stress and that scavenges of free radical could be key mechanism contribute to the cytoprotective effect of 3,4-di-*O*-caffeoyl quinic acid.

Key words: 3,4-di-O-caffeoyl quinic acid, DPPH radical, TBARS, HUVEC, endothelium-dependent vasodilatation

INTRODUCTION

Oxygen free radicals have been implicated in the pathophysiology of various vascular diseases such as ischemic-reperfusion injury and atherosclerosis[1]. Endothelial cells (ECs) play a major role in preserving the

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architecture and function of blood vessels[2], but they are vulnerable to free radicals because of their location in the vasculature. Mechanical damage to ECs is the direct result of peroxidation of cellular lipid and proteins[3]. Additional, functional damage of ECs can result from free radical inactivation of endothelium-derived relaxing factor, nitric oxide (NO)[4]. The consequent cellular disruption and inactivation of NO by free radicals can result in the loss of vascular regulation. Thus, the administration of free radical scavengers and antioxidants has been shown to provide protection of ECs against damage.

The whole plant of *Elephantopus mollis* H. B. K. (Compositae) has been used in Taiwanese folk medicine as antiviral, diuretic, and antibacterial agent for the treatment of pneumonia, arthralgia, hepatitis, and nephritis[5,6]. Recently, a phenolic constituent named as 3,4-di-*O*-caffeoyl quinic acid (Fig. 1) was isolated from *E. mollis* in our laboratory. In a large scale screening assay, we found that 3,4-di-*O*-caffeoyl quinic acid (0.1 to 1 μM) exhibited potent inhibitory effect to prevented copper-catalyzed oxidatiion of human low-density lipoproteins (LDL). Caffeoyl quinic acids are reported in several other natural sources and their antioxidant significance has been also discussed[7]. This antioxidative property would serve as an additional benefit given that 3,4-di-*O*-caffeoyl quinic acid may be useful in the prevention of various cardiovascular disorders. This study was aimed to evaluate whether 3,4-di-*O*-caffeoyl quinic acid could prevent free radical-induced impairment of vascular endothelial function.

MATERIALS AND METHODS

Isolation of 3,4-di-*O*-caffeoyl quinic acid

E. mollis was purchased from a Chinese herbal drug store in Taipei city, and were authenticated by Professor Jun-Chih Ou, Research Fellow, National Research Institute of Chinese Medicine. A voucher specimen (NRICM-05-010) of this herbal drug was deposited in the National Research Institute of Chinese

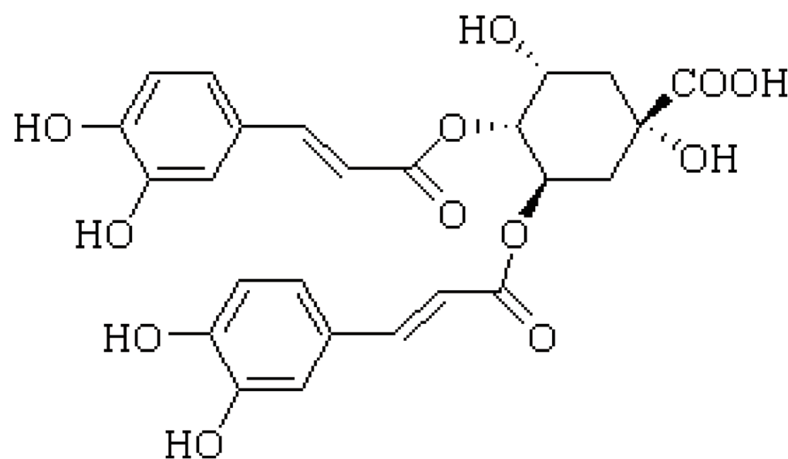

Fig. 1 The structure of 3,4-di-*O*-caffeoyl quinic acid.

Medicine, Taiwan, R.O.C. The dried whole plant of *E. mollis* (9.6 kg) was extracted with MeOH (2 x 100 L) at 60℃. The MeOH was removed *in vacuo* to give a dark brown MeOH extract which was suspended in 10 L H_2O to obtain H_2O-soluble and H_2O-insoluble portions. The H_2O-soluble portion was subjected to Diaion HP-20 chromatography, eluting with H_2O (10 L) and then with MeOH (10 L). The MeOH eluate was concentrated in vacuo, and the residue was separated over a Sephadex LH-20 column eluting with MeOH to yield 3,4-di-O-caffeoyl quinic acid (1.65 g). The structure of 3,4-di-*O*-caffeoyl quinic acid (Fig. 1) was determined by spectroscopic analyses and comparison of 1H- and 13C-NMR data published by Nishihzawa *et al.*[8].

DPPH scavenging assay

The free radical scavenging activity of 3,4-di-*O*-caffeoyl quinic acid was tested by employing 1,1-diphenyl-2-picrylhydrazyl (DPPH, Sigma) radical scavenging assay. Briefly, to a solution of DPPH (final concentration 200 μM) in absolute ethanol, an equal volume of the compound dissolved in ethanol was added at various concentrations. Ethanol was added to the control solution. Absorbance was recorded at 518 nm at room temperature[9]. Each experiment was performed at least in triplicate.

Measurement of membrane peroxidation by TBARS assay

The hydroxyl radical (•OH) is one of the strongest oxidants and can be produced by Fenton's reagent (H_2O_2/Fe^{2+})[10]. In this study, cultured human umbilical vein endothelial cells (HUVECs) were pretreated with 3,4-di-*O*-caffeoyl quinic acid for 2 h then membrane peroxidation was induced by incubated with H_2O_2 (2 mM)/$FeSO_4$ (10 μM) for 6 h at 37℃ in a 5% CO_2 atmosphere. At the end of the incubation period, cells were rinsed three times with cold PBS, scraped into 10 ml of fresh PBS and transferred to 15 ml culture tubes. The cells were then spun at 1000×g at 4℃ and the supernatant discarded. The final cell pellet was resuspended in 1 ml of PBS, of which 0.05 ml was aliquoted into eppendorf tubes for protein determination. The remaining 0.95 ml was used to assay for membrane lipid peroxidation. The extent of membrane lipid oxidation was determined by the thiobarbituric acid-reactive substances (TBARS) method and the absorbance was determined at 535 nm against a blank that contains all the reagents minus sample[11]. Malondialdehyde (MDA) obtained by acidification of malonaldehyde-bis-dimethylacetal (Aldrich, USA) was used as the standard for the quantification of TBARS. Results are expressed as nmol MDA/mg protein.

Culture of endothelial cells

Human umbilical vein endothelial cells (HUVEC) were obtained from ATCC and used at passages 2-4. The cells were grown in 75 cm^2 culture flasks (Falcon, Becton Dickinson, Lincoln Park, USA), filled with 10 ml of Ham's F-12 medium (Sigma, St. Louis, MO) containing 10% fetal calf serum (FCS) (Seromed, Berlin, Germany), 2 mM glutamine (Sigma), 30 μg/ml endothelial cell growth supplement (Sigma), 100 μg/ml heparin (Sigma), 100 U/ml penicillin (Sigma), and 100 μg/ml streptomycin (Sigma). The flasks were

incubated at 37℃, 100% humidity and 5% CO_2. The medium was refreshed every 2 days. At the beginning of each experiment the cells were detached by 0.01% trypsin/EDTA (Sigma). The trypsin was inactivated by dilution with PBS, and the cells were washed and counted. All culture experiments related to cell growth were carried out in six well microplates (Falcon, Becton Dickinson, Lincoln Park, USA), grown for 3 d, and then used for the study.

Induction of free radical-mediated cell injury and analysis of cytotoxicity

HUVEC cells ($2x10^6$ cells/well) in 6-well plates were incubated with various concentrations of 3,4-di-*O*-caffeoyl quinic acid for 2 h at 37℃. Oxidative stress was then initiated by the co-addition of H_2O_2 (1 or 2 mM) and 10 μM $FeSO_4$ and carried out for another 2 hr[12]. Cytotoxicity was assessed by Alamar Blue assay.

Alamar Blue assay

The Alamar Blue colorimetric growth indicator (Biosource International, Camarillo, CA) was used to measure cytotoxicity due to oxidative stress on endothelial cells[13]. It is a nonisotopic colorimetric assay used to measure quantitatively the viable cells in culture. HUVEC grown to 70–80% confluence were trypsinized, then seeded into six well microplates at 5000 cells per well in a 4 ml volume and allowed to form a monolayer overnight. Cell viability was assessed at 2 h after initiation of oxidative stress versus PBS controls in the absence or presence of 3,4-di-*O*-caffeoyl quinic acid. In the following study, fresh medium containing Alamar Blue growth indicator dye (10%, v/v) was added for another 4h-incubation at 37℃. The change in color could be monitored with an ELISA reader at 620 nm. Cell viability correlates with optical density. Wells containing medium and Alamar Blue dye without cells were used as blanks. In each case, the experiments were performed in duplicate. All experiments were repeated at least twice with similar results. The mean absorbance for the duplicate cultures of each exposure treatment was calculated and the mean blank value was subtracted from these. Cell viability in control medium without any treatment was represented as 100%.

Preparation of aortic rings and induction of free radical-induced vascular endothelium dysfunction

All experiments were performed in accordance with the Guidelines for Animal Experiments of the National Research Institute of Chinese Medicine. Thoracic aorta obtained from male Sprague-Dawley rat (250-300 g) was cut into ring and mounted in organ bath filled with oxygenated (95% O_2/5% CO_2) Krebs' solution[14]. After the aortic rings were randomized to receive 3,4-di-O-caffeoyl quinic acid for 2 h, Fenton's reagent was added to the organ bath for 30 min to induce free radical injury[12]. Then, sub-maximal contraction was achieved by addition of 0.3 μM phenylephrine (PE, Sigma) and cumulative relaxation curves to acetylcholine (ACh, Sigma) were obtained to assess endothelial function. Oppositely, sodium nitroprusside (Sigma) was used to assess the endothelium-independent relaxant function.

Statistical analyses

All values in the figures and table represent means±SEM. Data were analyzed by one-way analysis of variance (ANOVA) followed by *post-hoc* Dunnett's t test for multiple comparisons. Concentration dependency was analyzed by simple linear regression analyses of the dose-response relationship and testing the statistical significance of the regression slope by Student's t test. Values of $P < 0.05$ were considered significant.

RESULTS

The radical scavenging activity of 3,4-di-*O*-caffeoyl quinic acid was estimated by reactivity with DPPH. The absorbance of DPPH did not change obviously over 75 min observation in the absence of antioxidants. DPPH decolorization was significantly increased by 3,4-di-*O*-caffeoyl quinic acid in a concentration-dependent manner (Fig. 2). When 30 μM of 3,4-di-*O*-caffeoyl quinic acid was added to the mixture the absorbance decreased quickly. After 75 min, DPPH reduced reached 67 and 100% in the presence of 10 and 30 μM 3,4-di-*O*-caffeoyl quinic acid, respectively. Natural phenolic compound resveratrol was used as a reference antioxidant and compared with 3,4-di-*O*-caffeoyl quinic acid. Table 1 showed that resveratrol, a natural phenolic compound, was less effective than 3,4-di-*O*-caffeoyl quinic acid to quench DPPH radical at the concentration of 10 and 30 μM when measured at 75 min.

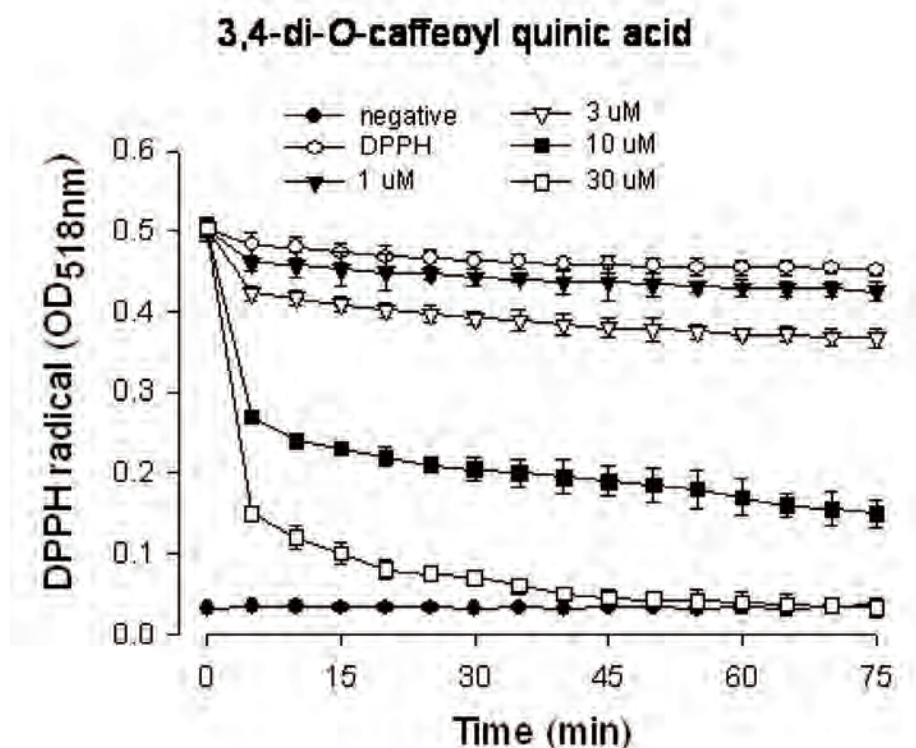

Fig. 2 DPPH radical scavenging activities of 3,4-di-O-caffeoyl quinic acid. All values are means ± SEM from five independent experiments.

Membrane lipid peroxidation in vascular endothelial cells was induced by incubation with Fenton's reagent as described in materials and methods. As shown in Fig. 3 (open bars, PBS control), no significant change in TBARS background level was noted when 3,4-di-*O*-caffeoyl quinic acid alone was incubated with HUVECs. Administration of Fenton's reagent for 6 h induced about 5-fold increases of TBARS values. When cells were pretreated with 3,4-di-*O*-caffeoyl quinic acid (0.5, 1, 5, and 10 μM) for 2 h, a concentration-dependent reduction of TBARS formation was observed (Fig. 3, hatched bars) with a significant effect was noted even at concentration as low as 0. 5 μM ($P < 0.05$).

Table 1. Comparison the DPPH radical scavenging activities of 3,4-di-*O*-caffeoyl quinic acid and resveratrol.

% Reduction of DPPH radical		
Concentration (μM)	3,4-di-O-caffeoyl quinic acid	Resveratrol
10	67.2±0.5	25.2±0.8
30	100.0±1.1	66.4±1.1

All values are mean±SEM from five independent experiments.

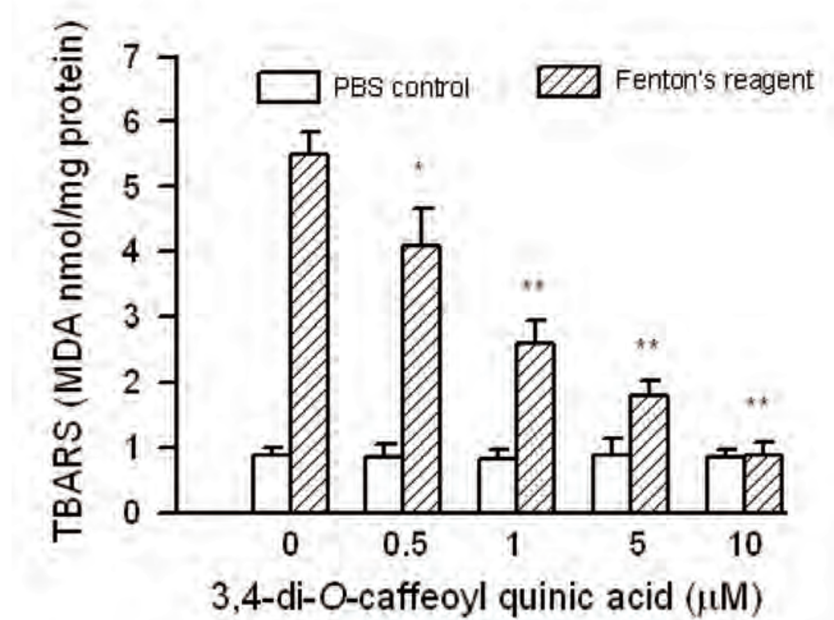

Fig. 3 Effect of 3,4-di-*O*-caffeoyl quinic acid on $H_2O_2/FeSO_4$-mediated membrane lipid oxidation in cultured human umbilical vein endothelial cells (HUVEC) as determined by the thiobarbituric acid-reactive substances (TBARS) method. Cells were pretreated with 3,4-di-*O*-caffeoyl quinic acid for 2 h then incubation with H_2O_2 (2 mM)/FeSO4 (10 μM) for another 6 hr. All values are means ± SEM of triplicate samples from five independent experiments. * $P < 0.05$ and ** $P < 0.01$, as compared to samples receiving $H_2O_2/FeSO_4$ alone.

Reductions in cellular viability decreased Alarma Blue absorbance. We found that incubation of 1 and 2 mM of H_2O_2 (in the continuous presence of 10 μM $FeSO_4$) with HUVECs evoked striking decrease the cell viability by 27% and 46%, respectively (Fig. 4, open bars). 3,4-Di-*O*-caffeoyl quinic acid alone did not induce any cellular toxicity, nevertheless, concentration-dependently reversed the cytotoxicity caused by Fenton's reagent (Fig. 4, hatched bars).

Acetylcholine (ACh), an endothelium-dependent vasodilator, was employed in this study to test the endothelial integrity. In control aortic rings precontracted with PE, ACh (1 μM-10 μM) produced a concentration-dependent vasodilatation with a maximal response of 85±4% (Fig.5, circles). One mM of H_2O_2 (in the continuous presence of 10 μM $FeSO_4$) was chosen to induce appropriate free radical injury. Results showed that the endothelium-dependent vasodilatation to acetylcholine was significantly destroyed after exposure to H_2O_2/$FeSO_4$: maximal relaxation caused by 10 μM acetylcholine was reduced to 41±3% (Fig.5, triangles). However, there was no difference in endothelium-independent vasodilatation to sodium nitroprusside (0.1 μM) between control vessels and those exposed to H_2O_2/$FeSO_4$ (97.2±6.3 vs. 98.1±5.5%). The present study showed that 3,4-di-*O*-caffeoyl quinic acid alone, at the concentrations used (5 and 10 μM), did no modify the basal vascular tone, the vasoconstriction response to PE, the original vasorelaxant response to acetylcholine, nor produce direct

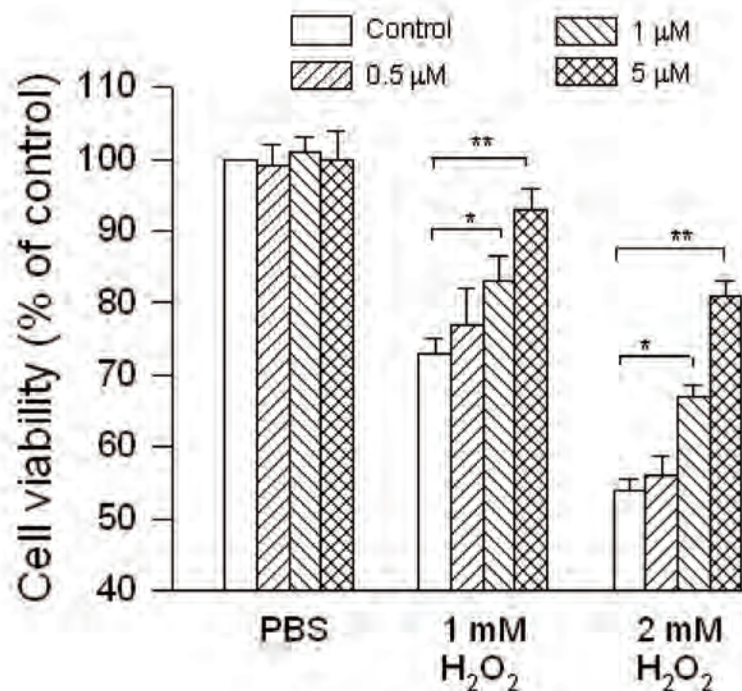

Fig. 4 Effect of 3,4-di-*O*-caffeoyl quinic acid on H_2O_2/Fe^{2+}-induced cytotoxicity in cultured human umbilical vein endothelial cells (HUVEC). Cells were pre-incubated with 3,4-di-*O*-caffeoyl quinic acid for 2 h, and then exposed to H_2O_2 (1 and 2 mM) in the continuous presence of FeSO4 (10 μM) for 2 hr. Cell viability was measured by Alamar blue assay as described in materials and methods. All values are means ± SEM of triplicate samples from five independent experiments. * $P < 0.05$ and ** $P < 0.01$, as compared to samples receiving H_2O_2/Fe^{2+} alone.

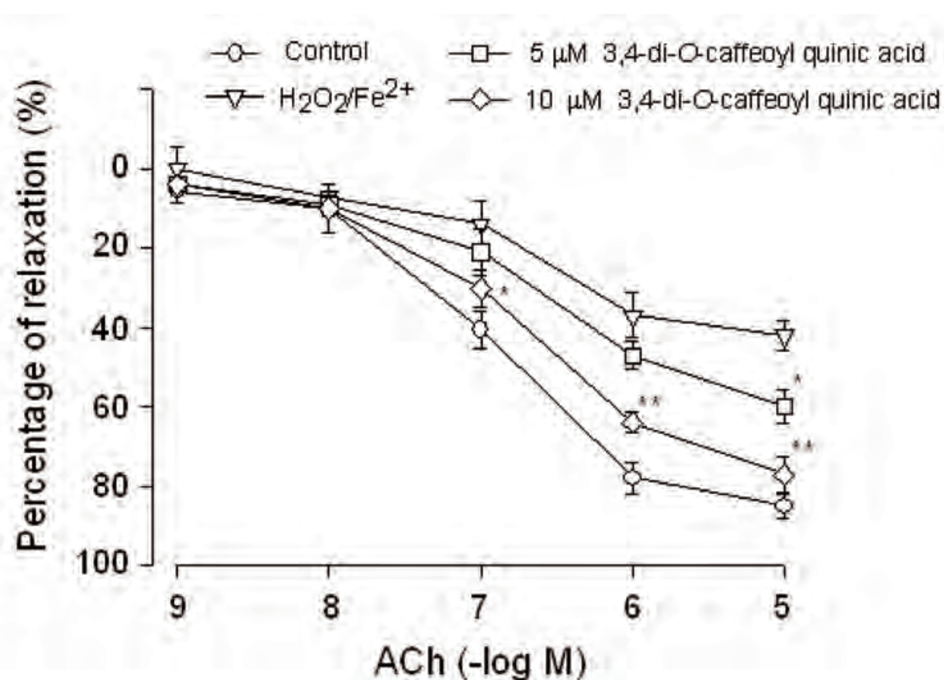

Fig. 5 Effect of 3,4-di-*O*-caffeoyl quinic acid on H_2O_2/Fe^{2+}–mediated impairment of endothelium-dependent relaxation to acetylcholine (ACh) in phenylephrine-precontracted aortic rings. Vascular rings were pre-incubated with 3,4-di-*O*-caffeoyl quinic acid for 2 h then exposed to H_2O_2 (1 mM) in the continuous presence of $FeSO_4$ (10 μM). All values are means ± SEM of data from eight independent experiments. * $P < 0.05$ and ** $P < 0.01$, as compared with $H_2O_2/FeSO_4$–challenged vascular beds before and after 3,4-di-*O*-caffeoyl quinic acid treatment.

vasodilatation (data not shown). However, pretreated the vessels with 3,4-di-*O*-caffeoyl quinic acid 2 h prior to $H_2O_2/FeSO_4$ caused apparently and concentration-dependently reverse of vascular hyporeactivity to acetylcholine (Fig. 5, squares and diamonds).

DISCUSSION

Recently, much attention has been focused on the protective function, especially antioxidative effect, of naturally occurring compounds and on the mechanisms of their action. Phenolic compounds have been considered to play an important role as dietary antioxidants for the prevention of oxidative damage in living systems. The antioxidant capability of these phenolic compounds is widely reported in the literature. In particular, it has been shown that these natural compounds show scavenging activity against superoxide anion radical ($O_2^{\bullet -}$), hydroxyl radical (•OH), singlet oxygen ($^1O_2$) and hydrogen peroxide[15,16]. There are considerable evidences that these free radicals induce oxidative damage to biomembranes. This damage causes atherosclerosis, aging, and several other diseases and antioxidants possess an important role in preventing these free radical-induced diseases[17,18].

In this study, the effects of 3,4-di-*O*-caffeoyl quinic acid, a phenolic compound isolated from E. mollis, on radical scavenging activities and prevention of oxidative stress-induced membrane lipid peroxidation were determined. 3,4-Di-*O*-caffeoyl quinic acid owned an ortho-dihydroxy phenyl ring, a caffeoyl moiety is known as efficient free radical scavenger[7]. Indeed, we found 3,4-di-*O*-caffeoyl quinic acid was effective to quench DPPH radical and had a strong protective action against oxygen free radical induced peroxidative damage to biomembranes as measured by TBARS formation.

Our results also showed that 3,4-di-*O*-caffeoyl quinic acid possess protective activity against Fenton's reagent (H_2O_2/Fe^{2+})-mediated harmful effects in culture endothelial cells and prevents H_2O_2/Fe^{2+}-induced impairment of endothelium-dependent relaxation in isolated rat aorta. The role of nitric oxide (NO) in endothelium-dependent relaxation is well known[19]. Endothelium-dependent agonists such as ACh stimulate NO synthase (eNOS) to release NO which results in relaxation by acting on soluble guanylate cyclase (sGC) to synthesize cGMP[20]. In blood vessels, Fenton's reagent (H_2O_2/Fe^{2+}) induces oxidative stress by generating hydroxyl radical (•OH) and inactivation of NO[21]. Phenols owned antioxidant properties to scavenge free radicals by electron transfer. A possible explanation that 3,4-di-*O*-caffeoylquinic acid prevents H_2O_2/Fe^{2+}-induced impairment of endothelial function may be attributed to electron transferring efficiency of the phenolic hydroxyl group in 3,4-di-*O*-caffeoylquinic acid.

In summary, the present study revealed that 3,4-di-*O*-caffeoyl quinic acid exhibits radical scavenging activities, and provides a protective effect against cytotoxicity induced by free radicals in vascular endothelial cells. The antioxidant and cell protection action of 3,4-di-*O*-caffeoyl quinic acid, therefore, may be useful in developing new agent against oxidative stress diseases. Since the oxidative modification of biomembrane plays an important role in the pathogenesis of atherosclerosis, 3,4-di-*O*-caffeoyl quinic acid may also help to reduce the risk of atherosclerosis, not only by protecting membrane lipids from oxidative modification but also by reducing free radical-induced endothelial injury and/or dysfunction. However, further studies are needed to unravel exactly under the cellular and molecular mechanisms or proper *in vivo* models for underlie the various pharmacological actions of 3,4-di-*O*-caffeoyl quinic acid.

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REFERENCES

1. Prasad K, Kalra J. Oxygen free radicals and hypercholesterolemic atherosclerosis: Effects of vitamin E. Am Heart J 1993; 125: 958-73.
2. Luscher TF. The endothelium as a target and mediator of cardiovascular disease. Eur J Clin Invest 1993; 23:

670-85.

3. Davies PF, Tripathi SC. Mechanical stress mechanisms and the cell: an endothelial paradigm. Cir Res 1993; 72: 239-45.
4. Rubanyi GM, Vanhoutte PM. Superoxide anions and hyperoxia inactivate endothelium-derived relaxing factor. Am J Physiol 1986; 250: H822-H827.
5. Lin CC, Yen MH. Development of natural crude drug resources from Taiwan (IX)—The pharmacognostical studies on the crude drug "Ding-kia-u". J Chin Med 1992; 2: 33-49.
6. Lin CC, Tsai CC, Yen MH. The evaluation of hepatoprotective effects of Taiwan folk medicine "Teng-Khia-U (Elephantopus mollis)". J Ethnopharmacol 1995; 45: 113-23.
7. Nardini M, D'Aquino M, Tomassi G, Gentili V, Felice MD, Scaccini C. Inhibition of human low-density lipoprotein oxidation by caffeic acid and other hydroxycinnamic acid derivatives. Free Radical Biol Med 1995; 19: 541-52.
8. Nishizawa M, Izuhara R, Kaneko K, Koshihara Y, Fujimoto Y. 5-Lipoxygenase inhibitors isolated from Gardeniae Fructus. Chem Pharm Bull 1988; 36: 87-95.
9. Andreadou I. Rekka EA, Demopoulos VJ, Bijloo GJ, Kourounakis PN. Synthesis, antioxidant and anti-inflammatory activity of novel substituted ethylenediamines and ethanolamines. A preliminary quantitative structure-activity relationship study. Arzneimittel-Forschung 1997; 47: 643-7.
10. Walling C. Fenton's reagent revisited. Acc Chem Res 1975; 8: 125-31.
11. Areias FM, Rego AC, Oliveira CR, Seabra RM. Antioxidant effect of flavonoids after ascorbate/Fe2+-induced oxidative stress in culture retinal cells. Biochem Pharmacol 2000; 62: 111-8.
12. Tsai JY, Chou CJ, Chen CF, Chiou WF. Antioxidant activity of piperlactam S: prevention of copper-induced LDL peroxidation and amelioration of free radical-induced oxidative stress of endothelial cells. Planta Med 2003; 69: 3-8.
13. Hein M, Ernst M, Moller F, Regensburger D. Gene transfer into rat heart-derived endothelial cells. Eur J Cardio-thoracic Surg 1998; 13: 460-6.
14. Chiou WF, Lin JJ, Chen CF. Andrographolide suppresses the expression of inducible nitric oxide synthase in macrophage and restores the vasoconstriction in rat aorta treated with LPS. Br J Pharmacol 1998; 125: 327-34.
15. Cui K, Luo X, Xu K, Murthy MRV. Role of oxidative stress in neurodegeneration: recent developments in assay methods for oxidative stress and nutraceutical antioxidants. Prog Neuropsychopharmacol Biol Psychiatry 2004; 28: 771-99.
16. Halliwell B, Gutteridge JM. Role of free radicals and catalytic metal ions in human disease: an overview. Methods Enzymol 1990; 186: 1-85.
17. Biesalski HK. Free radical theory of aging. Curr Opinion Clin Nutr & Metabolic Care 2002; 5: 5-10.
18. Nayak DU, Karmen C, Frishman WH, Vakili BA. Antioxidant vitamins and enzymatic and synthetic oxygen-derived free radical scavengers in the prevention and treatment of cardiovascular disease. Heart Dis 2001; 3:

28-45.

19. Palmer RMJ, Ferrige AG, Moncada S. Nitric oxide accounts for the biological activity of endothelium-derived relaxing factor. Nature 1987; 327: 524-6.
20. Rapoport RM, Murad F. Agonist-induced endothelium-dependent relaxation in rat thoracic aorta may be mediated through cGMP. Circ Res 1983; 52: 352-7.
21. Saıag B, Shacoori V, Bodin P, Pape D, Allain H, Burnstock G. Free radical involvement in endothelium-dependent responses of the rat thoracic aorta in moderate hypoxic conditions. Eur J Pharmacol 1999; 372: 57-63.

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3,4-DI-*O*-CAFFEOYL QUINIC ACID 保護血管內皮細胞對抗氧化性損傷及修復內膜依賴性血管舒張反應

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本文研究地膽草 (Elephantopus mollis H. B. K.) 酚類成份3,4-di-*O*-caffeoyl quinic acid 對於氧化壓力所造成血管內皮細胞損傷的保護作用。結果顯示3,4-di-*O*-caffeoyl quinic acid (1 to 30 μM)可清除DPPH自由基、且活性大於resveratrol。由TBARS的實驗發現3,4-di-*O*-caffeoyl quinic acid也可有效抑制 Fenton's reagent ($H_2O_2/FeSO_4$) 引起的細胞膜氧化損傷。此外，3,4-di-*O*-caffeoyl quinic acid不但可以減低 Fenton's reagent ($H_2O_2/FeSO_4$)所造成的人類臍靜脈血管內皮細胞 (human umbilical vein endothelial cells, HUVEC) 死亡，也可以使胸主動脈血管遭破壞的內膜依賴性舒張反應得以恢復。以上結果顯示3,4-di-*O*-caffeoyl quinic acid 可對抗氧化性損傷，而自由基清除作用可能就是此成份的細胞保護機轉之一。

關鍵詞：3,4-di-*O*-caffeoyl quinic acid；DPPH自由基；TBARS；人類臍靜脈血管內皮細胞；內膜依賴性血管舒張反應

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