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Vasorelaxant Effects of Ethanolic Extract from *Cydonia oblonga* Mill. Leaves on Isolated Rat Thoracic Aorta and Potential Mechanism of Action

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Abstract

Objective: *Cydonia oblonga* Mill. leaves ethanolic extract (CydOL-EE) has shown different cardioprotective effects. However, no previous studies investigated its direct effect on the vascular smooth muscle tone. Therefore, the study aimed to test the potential vasodilator activity of CydOL-EE in *ex-vivo* rat thoracic aorta preparations with an additional investigation of its mechanistic effects. **Methods:** CydOL-EE phytochemical profile was first investigated by HPLC-DAD-ESI-MS/MS and then tested for the vasorelaxation/vasoreactivity effects in rat aortic rings. The NO synthase inhibitor *N*(ω)-nitro-L-arginine methyl ester (L-NAME) and cyclic guanosine monophosphate inhibitor 1H-[1,2,4]Oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) were used to explore of the involvement of NO-dependent pathways. **Results:** Chromatographic analysis of CydOL-EE revealed the presence of six flavonols and seven hydroxycinnamic acids. Moreover, CydOL-EE showed a decrease in vasoreactivity caused by dose-dependent phenylephrine (PE) (Control, Emax = 104.29 ± 3.67 vs CydOL-EE, Emax = 70.73 ± 3.67, $P < .0001$) and a direct relaxing activity to precontraction with PE (Emax = 79.63 ± 3.67%). These responses were abolished during e-NOS inhibition, demonstrating that the mechanism of action was predominately controlled by the participation of an endothelium-dependent system. **Conclusion:** The results of our study show that CydO-EE demonstrates vasorelaxation and reduction of vasoreactivity through a NO-dependent pathway. These findings provide scientific evidence for further understanding of CydOL-EE use in the treatment of cardiovascular disease.

Keywords

Cydonia oblongata Mill., leaves ethanolic extract, aorta, vasorelaxation, vasoreactivity, nitric oxide

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Introduction

C. oblonga Mill., a member of the *Rosaceae* family, is primarily grown in the Turkey Region presently, although it originated in the Trans-Caucasus region.[1] Its green, elliptical leaves are 5–10 cm long and have white hairs on the outside.[2] Based on the phytochemical examination, the main constituents of *C. oblonga* Mill. were flavonoids, α and β ionol glycosides, organic acids, and phenolic compounds.[3,4] *C. oblongata* Mill. (CydOL) ethnobotanical uses are in the food industry, culinary uses and medicinal uses for gastrointestinal, respiratory, cardiovascular and infectious diseases.[5] However CydOL extracts have been demonstrated to have pharmacological properties that are potentially useful in the treatment of diabetes, cough, and bronchitis.[6,7] Additional research using in vitro models demonstrated its antihemolytic and free radical scavenging activity, with IC_{50} mean values of 30.7 and 24.3 g/mL of methanolic leaf extract,[8] antibacterial[9] and antioxidant effects with EC_{50} mean value of 21.6 μg/mL of ethanolic leaf extract,[10] anticancer activity in renal and colon cells with $IC_{50} = 239.7$ μg/mL of methanolic leaf extract.[11] Additionally, there has been a demonstration *in vivo* of significant protection against stomach ulcer size and severity, pepsin activity and myeloperoxidase activity of methanolic and aqueous leaf extract,[12] well as *in vivo* hypolipidemic and hypoglycemic activity or significant reduction of

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glucose and TC,TG, LDL-C levels with ethanolic leaf and fruit extracts,[13,14] cardioprotective effects[6] and hypotensive effects in the rat renal hypertensive models and reduction of blood rheology with −27 mm Hg with ethanolic leaf extract.[15] Furthermore, phenolic compounds found in CydOL-EE have demonstrated cardioprotective effects against the metabolic syndrome and its associated diseases, including diabetes, obesity, dyslipidemia, ischemia/reperfusion injury, heart failure, and atherosclerosis, as well as lowering blood pressure and lowering the risk of coronary heart complications through antioxidant and anti-inflammatory properties, as well as by supporting mitochondrial function in cardiac cells.[16-19]

However, the CydOL plant extract, such as seeds methanol-aqueous extract (0.003-10 mg/mL) has been demonstrated to have dose-dependent efficacy in releasing *in vitro* spasms in the ileum (EC_{50} value of 0.73 mg/mL from ACh and EC50 value of 0.86 mg/mL from KCl) and dilating the in *vitro* respiratory smooth muscle tone, such as tracheal rings of rabbits (EC value of 0.41 mg/mL from KCl and EC_{50} value of 0.94 mg/mL from CCh).[20]

The NO pathway has a very important role in the endothelium-dependent vasorelaxation responses and CVD; therefore endogenous endothelium-dependent NO production lies mainly in the activation of endothelial NO synthase enzyme which activates the cyclic GMP for inducing vascular smooth muscle vasodilation.[21,22] However, there is a lack of previous reports that show the *in vitro* vasodilatory action of the CydOL-EE and its related mechanistic effects in rat aortic tissue. Therefore, the goal of this study was to perform an investigation of the vasorelaxant effects of CydOL extract and its related endothelium dependent mechanism of action in the isolated rat aorta.

Results and Discussion

CydOL-EE Phytochemical Profile

The CydO-EE (extraction yield 3.8% *w*/*w*) phytochemical profile was investigated by HPLC-DAD-ESI-MS/MS. Chromatographic analysis revealed the presence of six flavonols (quercetin 3-*O*-rutinoside, 3-*O*-glucoside, 3-*O*-ramnoside and 3-*O*-*p*-coumaroylglucoside, and kaempferol 3-*O*-rutinoside and 3-*O*-p-coumaroylglucoside) and seven hydroxycinnamic acids (3-*O* and 5-*O* caffeoylquinic and feruloylquinic acids, 4-*p*-coumaroylquinic acid and *p*-coumaroylquinic acid isomer, and 3,5-Dicaffeoyl quinic acid). The chemical structures of identified compounds are presented in Figure 1, whereas typical chromatogram obtained at 330 nm and characteristic UV spectra of each class of polyphenolic compounds are

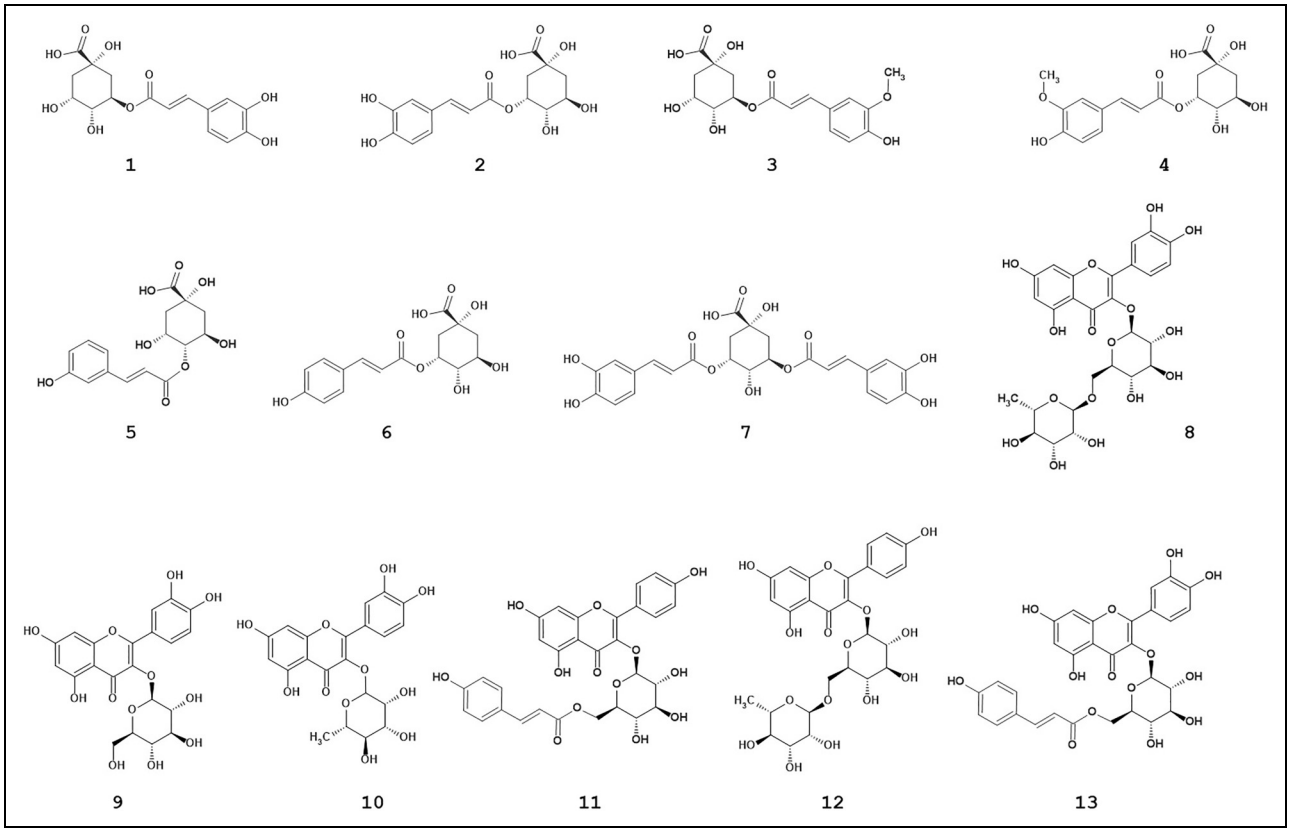

Figure 1. Chemical structures of 3-*O*-caffeoyquinic acid (1), 4-*O*-*p*-Coumaroylquinic acid isomer (2), 5-*O*-caffeoylquinic acid (3), 3-*O*-feruloylquinic acid (4), 4-*O*-*p*-coumaroylquinic acid (5), 3,5-*O*-dicaffeoyl quinic acid (6) quercetin-3-*O*-rutinoside (7), 5-*O*-feruloylquinic acid (8), quercetin 3-*O*-(3′-*O*-*p*-coumaroyl)-glucoside (9), quercetin-3-*O*-glucoside (10), quercetin-3-*O*-rhamnoside (11), kaempferol 3-*O*-rutinoside (12) and kaempferol 3-*O*-(4″-*O*-*p*-coumaroyl)-glucoside (13).

presented in Fig S1. Structural characterization data (t_R, UV max, MS data) and content (mg/100 g) of phenolic compounds are presented in Table S1. The results from phytochemical profile and content are in good agreement with those recently reported in the literature by Zhang et al (2021) for *C. oblonga* leaves methanol extract based on UHPLC-QTOF-MS data.[23]

Vasorelaxant Effect of CydOL-EE and its Effect on Aortic Vasoreactivity

According to our findings, there was a concentration-dependent relaxing effect (Emax = 79.63 ± 3.67%) for CydOL-EE (0.01-0.1 mg/mL). The inhibition of eNOS and cGMP pathways affected this pharmacological activity; L-NAME + CydOL-EE: Emax = 3.56 ± 0.85%, ODQ + CydOL-EE: Emax = 5.66 ± 0.84%, respectively, contributed to a decrease in the vasorelaxation effect of CydOL-EE (Figure S2A-C, Table S2).

Furthermore, a decrease in vasoreactivity was observed upon incubating tissues with CydOL-EE (0.1 mg/mL), which was found to significantly reduce the maximal effect of the concentration-response curve of cumulative doses of PE (0.01-10 μM) in aortic rings (control Emax = 104.29 ± 3.67 vs CydOL-EE Emax = 70.73 ± 3.67, P < .0001). This demonstrated the antiadrenergic effects of CydOL-EE via alpha-1 adrenergic receptors in the vascular smooth muscle.[24] Moreover, eNOS inhibition fully eliminated this response (L-NAME + CydOL-EE: Emax = 113.51 ± 43.50; ODQ + CydOL-EE: Emax = 112.51 ± 2.50) (Figure S3, Table S3). Because the ability of CydOL-EE to dilate blood vessels was completely eliminated by eNOS and cGMP inhibitors, these results clearly demonstrate the endothelium-dependent origin of the observed vasorelaxant effect, which is mediated mainly by the activation of NO pathways. This suggests that endothelium-dependent NO pathways were at play.

A number of studies have shown that CydOL has antihypertensive effects *in vivo* in renal hypertensive rats[15] and has an effect on blood rheology and rat renal hypertension,[14] lowers hypertension in spontaneously hypertensive rats,[25] and lowers systolic, diastolic, and mean arterial pressures in rats and mice,[26] there is currently insufficient evidence to support the role of CydOE in direct vascular smooth muscle tone. While reports on non-vascular smooth muscles, such as bronchodilators, antispasmodics, or smooth muscle relaxation in the colon, have only been found in relation to CydO seed extract which has a different chemical profile[20] our study provides the first empirical evidence of CydOL-EE's capacity to relax the blood vessels in aortic rings. Phenolic compounds found in CydOL-EE have demonstrated properties that aid in the management and avoidance of vascular disorders.[27-30] The direct vasorelaxant impact that is reliant on the cyclic NO / GMP pathway[31,32] and the decrease in PE-induced contractions, respectively, alpha1 receptors, are further indications of these effects.[33] The results of these investigations corroborate our findings and shed insight on the function and importance of the phytochemicals present in CydOL-EE and partially explain the pharmacological effects of CydOL-EE on cyclic GMP, a vascular tone reliant on the NO pathway.

Experimental

See Supplemental Material.

Conclusions

The results of this study demonstrate that the vasorelaxant action of CydOL-EE is mediated by the endothelium-dependent NO pathway. Taken together, these findings might explain the effects of CydOL-EE on the vascular tone control. Additional *in vitro* and *in vivo* investigations are necessary to better distinguish the related mechanism of action beyond that demonstrated in this study. However, this study may pave the way for further investigation on the application of CydOL-EE in vascular tone control.

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Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Approval

This study was approved by the Research and Ethics Committee of the Faculty of Medicine at the University of Prishtina, Kosovo (Ref Nr.5181).

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Statement of Human and Animal Rights

All the experimental procedures involving animals were conducted in accordance with the Institutional Animal Care Guidelines of University of Prishtina, Kosovo and approved by the Research and Ethics Committee of the Faculty of Medicine at the University of Prishtina, Kosovo (Ref. Nr. 5181)

Statement of Informed Consent

There are no human subjects in this article and informed consent is not applicable.

Supplemental Material

Supplemental material for this article is available online.

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