

VASODILATOR EFFECT OF OLIVE LEAF AQUEOUS EXTRACT AND INVOLVMENT OF CYCLOOXYGENASE IN THE RAT AORTA

Karimi G, Imenshahidi M, Jafarizaveh E, Asgharian Rezaee M.

Medical Toxicology Research Center and Pharmacy faculty, Mashhad University of Medical Science, Mashhad, Iran;

Summary

We have investigated some mechanism of vasorelaxant effect of olive leaf aqueous extract in rat aorta ring. In the isolated aorta pre-contracted with phenylephrine cumulative concentration of extract induced vasorelaxation. Cyclooxygenase, NO and endothelium pathways was examined. Vasorelaxant effect of extract inhibited with Indomethacin but didn't affect with L-NAME, this effect also was endothelium-independent. the results suggest that vasorelaxant effect of extract induced via Cyclooxygenase pathway and may be there is an interaction between olive leaf extract and vasorelaxant prostanoids.

Key words: Aorta, olive leaf, endothelium, Nitric Oxide, Cyclooxygenase.

Introduction

Olea europaea belonging to the *oleaceae* family, grows in almost all of the Southern European Countries and throughout the entire Mediterranean region as for as Iran and beyond the Caucasus (1).

Olive is a small evergreen tree up to 10M high, with hoary, rigid branches and grayish bark. The fruit is a drupe about the size of damson, smooth, 2-celled, with a nauseous, bitter flesh, enclosing a sharp-pointed stone(1, 2, 3).

Olive leaf has been shown to contain: Iridoid monoterpenes, triterpenes, flavonoids, chalcones (1, 3) and recently investigated the digalactoside of poly-unsaturated diester of the glycerol (4).

Olea europaea preparations have been used widely in folk medicine in different countries. The medical parts of olive tree are the dried leaves, the oil extracted from the ripe drupes, and fresh branches containing leaves and clusters of flowers(1, 2, 3, 5, 6).

In folk medicine olive leaf have been used as diuretic, hypotensive, emollient and for treatment of arteriosclerosis, rheumatism, gout, diabetes mellitus, fever, inflammation of gallbladder, Icterus, Roemheld syndrome, gastrointestinal ulcers and kidney stones (1, 7, 8, 9, 10, 11)

Also in animal experimentation olive leaf has demonstrated hypotensive, anti arrhythmic and spasmolytic effect on the smooth muscle of the intestine, caused by terpenes and phenols components (1, 12, 13).

In previous study, vasodilator and hypotensive effect of olive leaf decoction has been investigated(13) but the mechanism is unknown.

In the present study, we aimed to investigate mechanism of vasodilator effect of live leaf aqueous extract.

Materials and Methods

Plant

Fresh olive leaf collected in September 2005 in Gorgan (Province of Golestan, Iran), dried in room temperature, and broke to pieces, then preparing aqueous extract.

Animal

Male wistar rats approximately 220-300 g, from animal room of Mashhad Pharmacy Faculty. The animals were housed in environment at 20-25°C, with a 12-h light: 12-h dark cycle. Start at 8:00 with free access to food and water.

Tissue preparation

The animal were anesthetized with intraperitoneal injection of ketamine (60 mg/kg) and xylazine(6 mg/kg), then thoracic aorta immediately removed, aorta placed in Krebs solution (containing (in mM)): NaCl, 118; NaHCO₃, 25; MgSO₄, 1.17; CaCl₂, 1.6; KH₂PO₄, 1.18, (+)-D-Glucose 11.1; KCl, 4.7) bulbed with mixture of 95% O₂ and 5% CO₂. After cleaning the tissue free of fat and other adhering tissue, the vessels were cut into 3mm long rings, with special care to avoid damaging the endothelium. The preparation were mounted on a pair of stainless-steel hooks; one of each was fixed to an isometric force transducer (Narcobiosystem, USA) connected to a polygraph

(Narcobiosystem, USA). Tissue were allowed to equilibrate under an optimum final force of 1.5 g for a period of 60 min, in a water-jacketed tissue bath (10 ml) containing oxygenated Krebs solution at 37°C (final pH of 7.4), renewing the buffer every 15 min.

The presence of functional endothelium was tested by relaxation response to cumulative concentration of acetylcholine in phenylephrine- precontracted rings. Also presence of functional Nitric Oxide pathway was tested by relaxation response to sodium nitroprusside (10^{-9} - 10^{-4} M) in phenylephrine-precontracted rings.

Experimental protocol

All of the following experiments were conducted on the aorta rings. To evaluate a possible relaxant action of olive leaf extract, the effect of cumulative concentration of extract (0.125, 0.25, 0.5, 1 mg/ml) on the phenylephrine (10^{-6}) precontracted aorta rings were analyzed for 4 series of experiment: (1) intact aorta rings; (2) endothelium – denuded aorta rings; (3) aorta rings incubated with L-NAME (10^{-4} M) 20 min before inducing contraction with phenylephrine; (4) aorta rings incubated with indomethacin (10^{-5} M) 20 min before adding phenylephrine.

Endothelium-denuded rings were prepared by removing endothelium by rubbing the lumen of aorta ring with a cotton thread, and removal was confirmed by the loss of relaxation to 1μ M acetylcholine.

Statistical analysis:

All results are presented as mean \pm SEM For the number of rats. One way ANOVA and Student's unpaired *t*-test were used for statistical analysis, followed by Tukey-Kramer as *pot hoc* test. Differences were considered significant at $P < 0.05$.

Results

1) Acetylcholine induced vasodilation

The cumulative addition of Ach (10^{-8} - 10^{-5}) to phenylephrine (PE) - precontracted aorta rings, caused a relaxation response. The maximum response (E_{\max}) was $76.27 \pm 6.3\%$

2) Effect of olive leaf extract on aorta ring in presence and absence of endothelium

In PE- precontracted aortic rings, cumulative concentration of extract (0.125, 0.25, 0.5, 1 mg/ml) applicated to intact and denuded aortic rings. The relaxation response was observed. This effect was concentration-dependent and endothelium-independent. In denuded endothelium E_{\max} was $41.03 \pm 2.76\%$, but non significant difference observed compare to intact endothelium (Fig. 1).

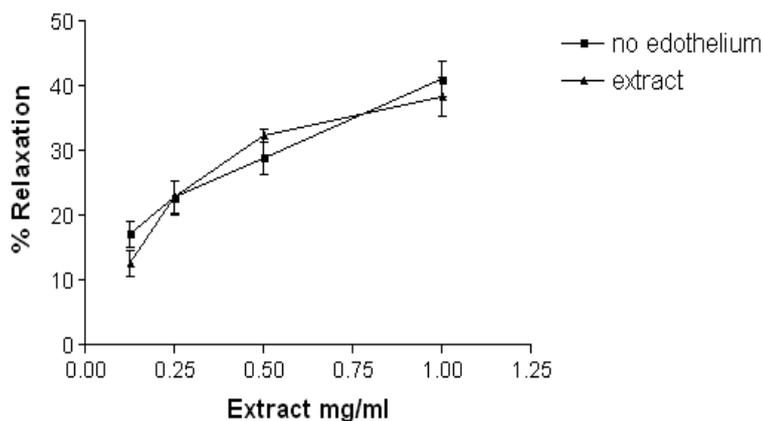


Figure 1: The relaxation of PhE (10^{-6} M) pre-contracted rat aorta rings in response to cumulative addition of the extract in presence and absence of endothelium. Data are presented as mean \pm SE , n :4.

3) Effect of olive leaf extract on aortic rings in presence of L-NAME

Endothelium-intact aortic rings were exposed to L-NAME (10^{-4} M) for 20 min prior to application of PE. Once the sustained contraction to PE was established, olive extract was added cumulatively to the bath. Fig (2) shows that pretreatment of aortic rings with L-NAME didn't decrease the relaxation induced by the extract (E_{max} : 45.53 ± 4.64)

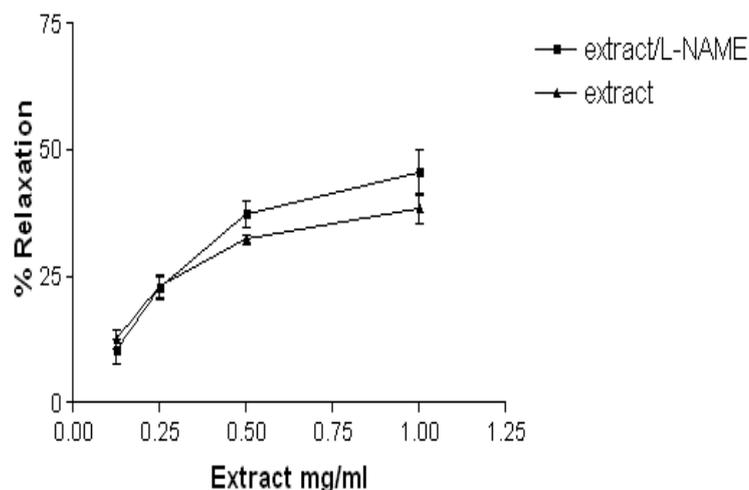


Figure 2: The relaxation of PE (10^{-6} M) pre-contracted rat aorta rings in response to cumulative addition of the extract in presence and absence of L-NAME (10^{-4} M). Data are presented as mean \pm SE , n :4.

4) Effect of olive leaf extract on aortic rings in the presence of Indomethacin

Indomethacin (10^{-5} M) applied in aortic rings 20 min prior to addition of PE (10^{-6} M) and then cumulative concentration of extract. Emax for pretreatment with indomethacin was 28.72 ± 2.08 . The result (Fig. 3) shows that indomethacin significantly reduced vasorelaxant effect of extract.

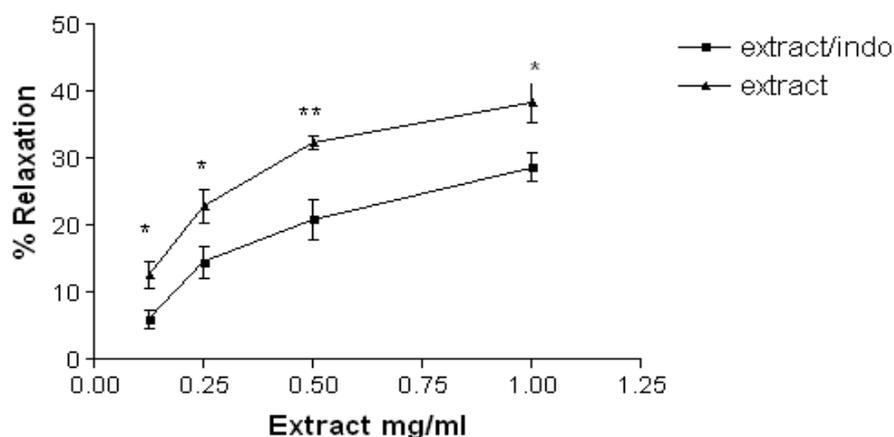


Figure 3: The relaxation of PE (10^{-6} M) precontracted rat aorta rings in response to cumulative addition of the extract in presence and absence of indomethacin (10^{-5} M). Data are presented as mean \pm SE, n :4.

Discussion

The results of this study showed that the aqueous olive leaf extract induced concentration-dependent relaxation in rat aorta and vasorelaxant effect of extract involved mainly endothelium-independent mechanism.

The effect of L-NAME was examined. Pre-treatment with L-NAME didn't diminish extract-induced relaxation in rat aorta, so the vasorelaxation effect isn't via NO pathway.

It has been showed that the decoction of olive leaf has vasorelaxant effect and this effect was endothelium independent(13). In this research, we evaluated the vasodilatory mechanisms of aqueous olive leaf extract and the role of NO and Cyclooxygenase pathway. In other series of our experiment, involvement of Cyclooxygenase in vasodilatory effect of extract was evaluated. Indomethacin decreased vasodilation; this finding showed a possible role of Cyclooxygenase pathway in observed vasodilation effect of olive leaf extract. These results provided evidence that prostanoids might contribute in vasodilation. The role of prostanoids in control of the vascular tone has been largely documented(14, 15, 16, 17, 18).

Among prostanoids, prostacyclin has been known as the prominent vasorelaxant agent(14, 15). Vascular prostacyclin is synthesized by both endothelial and smooth muscle cells of arteries(16, 17). The K_{ATP} role in vasodilatory effect of prostacyclin has been reported(18). COX inhibitors such as NSAIDs reduce prostanoid production and their effects(14).

Many chemical constituent have been identified and isolated from olive leaves (1, 3). A recent study indicated the presence of unsaturated fatty acid derivative in olive leaf(4). This component is di-galactoside of a poly unsaturated diester of glycerol. The poly-unsaturated acid that esterifies the glycerol is α -linolenic acid(3).

There are some studies about the vasodilatory effect of poly unsaturated fatty acid(19, 20, 21). Engler, et al, showed the omega-3 derivatives of unsaturated fatty acid (Docosahexanoic acid (DHA), Eicosapentaenoic acid (EPA)) induce relaxation in isolated aorta and this effect inhibited by indomethacin and gilbenclamid. The authors suggested that vasorelaxation role of DHA and EPA might be due to production of prostanoid that activate K_{ATP} channel (19, 20, 21, 22). Their effects were not dependent to endothelium and NO-pathway. Also vasorelaxant effect of alpha – linolenic acid as polyunsaturated fatty acid was shown previously (23).

Because of polyunsaturated fatty acid derivatives that have been identified in olive leaf, the vasorelaxation effect of olive leaf extract may be related to this component. Further experiments are presently underway to elucidate the active principles and mechanism of action.

In conclusion, this study reveals that aqueous olive leaf extract, elicit an endothelium-independent vasorelaxation. Cyclooxygenase pathway may be involved in such vasorelaxation. Olive leaf extract may be act as precursor of vasorelaxant prostanoids or induce endothelium-independent vasorelaxant agent. This finding also provides a mechanistic basis for traditional herb prescription for treatment of cardiovascular disease.

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References

1. Gruenwald J. PDR: for herbal medicines. 3th ed. Montvale: NJ: Thomson; 2004.
2. Dermarderosian A. The review of natural product. 1rd ed. Missouri: Fact and Comparisions; 2001.
3. Khan Y, Panchal S, Vyas N, Butani A, Kumar V. Plant Review. *Olea europaea*: A phyto-pharmacological review. *Phcog Rev.* 2007;7(1):114-8.
4. Bianco A, Mazzei RA, Melchioni C, et al. Microcomponents of olive oil. Part II: Digalactosyldiacylglycerols from *Olea europaea*. *Food Chem.* 1998;62(3):343-6.

5. Ebadi M. In pharmacodynamic basis of herbal medicine. 1rd ed. Boca Ratton: CRC press LLC; 2002.
6. Tunstall-Pedoe H, Kuulasmaa K, Mahonen M, et al. Contribution of trends in survival and coronary-event rates to changes in coronary heart disease mortality: 10-year results from 37 WHO MONICA project populations. Monitoring trends and determinants in cardiovascular disease. *Lancet*. 1999 May 8;353(9164):1547-57.
7. Ribeiro Rde A, Fiuza de Melo MM, De Barros F, Gomes C, Trolin G. Acute antihypertensive effect in conscious rats produced by some medicinal plants used in the state of Sao Paulo. *J Ethnopharmacol*. 1986 Mar;15(3):261-9.
8. Lawrendiadis G. CONTRIBUTION TO THE KNOWLEDGE OF THE MEDICINAL PLANTS OF GREECE. *Planta Med*. 1961;9(02):164-9.
9. Al-Khalil S. A Survey of Plants Used in Jordanian Traditional Medicine. *Pharmaceut Biol*. 1995;33(4):317-23.
10. Zargari A. Medical plants: Tehran University Publication; 1992.
11. Bellakhdar J, Claisse R, Fleurentin J, Younos C. Repertory of standard herbal drugs in the Moroccan pharmacopoea. *J Ethnopharmacol*. 1991 Dec;35(2):123-43.
12. Khayyal MT, el-Ghazaly MA, Abdallah DM, et al. Blood pressure lowering effect of an olive leaf extract (*Olea europaea*) in L-NAME induced hypertension in rats. *Arzneimittelforschung*. 2002;52(11):797-802.
13. Zarzuelo A, Duarte J, Jimenez J, Gonzalez M, Utrilla MP. Vasodilator effect of olive leaf. *Planta Med*. 1991 Oct;57(5):417-9.
14. Gryglewski RJ. Prostacyclin among prostanoids. *Pharmacol Rep*. 2008 Jan-Feb;60(1):3-11.
15. Norel X. Prostanoid receptors in the human vascular wall. *ScientificWorldJournal*. 2007;7:1359-74.
16. Frolich JC. Prostacyclin in hypertension. *J Hypertens Suppl*. 1990 Sep;8(4):S73-8.
17. Luscher TF, Barton M. Biology of the endothelium. *Clin Cardiol*. 1997 Nov;20(11 Suppl 2):II-3-10.
18. Jackson WF, Konig A, Dambacher T, Busse R. Prostacyclin-induced vasodilation in rabbit heart is mediated by ATP-sensitive potassium channels. *Am J Physiol*. 1993 Jan;264(1 Pt 2):H238-43.
19. Engler MB, Engler MM. Docosahexaenoic acid--induced vasorelaxation in hypertensive rats: mechanisms of action. *Biol Res Nurs*. 2000 Oct;2(2):85-95.
20. Engler MB, Engler MM, Ursell PC. Vasorelaxant properties of n-3 polyunsaturated fatty acids in aortas from spontaneously hypertensive and normotensive rats. *J Cardiovasc Risk*. 1994 Jun;1(1):75-80.
21. Engler MB. Effect of omega-3 fatty acids, docosahexaenoic and eicosapentaenoic, on norepinephrine-induced contractions. *Can J Physiol Pharmacol*. 1992 May;70(5):675-9.
22. Engler MB, Engler MM, Browne A, Sun YP, Sievers R. Mechanisms of vasorelaxation induced by eicosapentaenoic acid (20:5n-3) in WKY rat aorta. *Br J Pharmacol*. 2000 Dec;131(8):1793-9.
23. Blondeau N, Petrault O, Manta S, et al. Polyunsaturated fatty acids are cerebral vasodilators via the TREK-1 potassium channel. *Circ Res*. 2007 Jul 20;101(2):176-84.