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Blood pressure lowering effect of olive is mediated through calcium channel blockade

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Abstract

Olive (*Olea europea*) is used in traditional medicine as a remedy for hypertension. The aqueous–methanolic crude extract of *O. europea* fruit (OeF.Cr) was studied in anaesthetized rats and its possible mechanism was elucidated using isolated cardiovascular preparations. Intravenous administration of OeF.Cr produced a dose-dependent (30–100 mg/kg) fall in arterial blood pressure in normotensive anaesthetized rats. This effect remained unaltered in atropinized animals. In the *in vitro* studies OeF.Cr (0.1–3.0 mg/ml) inhibited spontaneously beating guinea-pig atria. Moreover, it relaxed K^+ and/or phenylephrine-induced contractions of rabbit aortic preparations over a dose range of 0.1–3.0 mg/ml, suggesting calcium channel blockade (CCB). The CCB effect was confirmed when pretreatment of the vascular preparations with OeF.Cr produced a dose-dependent rightward shift of the Ca^{2+} dose–response curves, similar to verapamil. These results suggest that the blood pressure lowering effect of olive is mediated through CCB, justifying its use in hypertension.

Keywords: *Olive, antihypertensive, calcium antagonist, rats*

Introduction

Olea europea (olive), locally known as 'zaitoon', is a small evergreen tree, native in all probability to parts of southern Europe and Asia Minor. It is cultivated on the shores of the Mediterranean, and also in California, Australia and other parts of the world (Nadkarni 1976). The plant has a folkloric reputation as an aphrodisiac, emollient, laxative, nutritive, sedative and tonic, and is used to treat abdominal colic (spasm), alopecia, paralysis, rheumatic pains, sciatica (Usmanghani et al. 1997) and hypertension (Ziyyat et al. 1997). Olive oil and fruit are edible (Nadkarni 1976).

Phytochemical studies revealed the presence of numerous bioactive compounds: alpha-tocopherol, apigenin, arabinose, beta-carotene, caffeic acid, catechin, choline, elenolide, esculin, estrone, fat, fiber, iron, linoleic-acid, luteolin, mannitol, oleaniolic acid, oleuropein, olivine, pactin, palmitic acid, rutin, squalene, tannins and verbascoside (Duke 1992).

Olive oil has been reported to reduce the incidence of coronary heart diseases (Keys 1987). The leaves of *O. europea* are known to possess hypocholesterolemic

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(De Pasquale et al. 1991), hypotensive (Lasserre et al. 1983) and vasodilator (Zarzuelo et al. 1991) activities. However, these are preliminary studies with the precise mode of action remaining to be elucidated. Despite the fact that olive fruit has been commonly used for medicinal purposes, it has not been widely studied for its biological activities.

In this investigation we report that the olive fruit extract lowers blood pressure through calcium antagonistic activity, and thus there is a sound mechanistic basis for its folkloric use in hypertension.

Materials and methods

Plant material and preparation of crude extract

Fresh fruits of *O. europea* were bought from a local market in Dhaka (Bangladesh) in December 2002, and the sample voucher (OE-FR-01-03-43) has been submitted to the herbarium of Department of Biological and Biomedical Sciences, Aga Khan University, Karachi. A total 603 g of fresh juicy fruits were sliced and soaked in 70:30% methanol and water for 3 days with occasional shaking. The initial preparation was filtered through a muslin cloth, then through filter paper. This procedure was repeated twice and the combined filtrate was evaporated through rotary evaporator under reduced pressure to a thick, semi-solid, dark-yellow mass (i.e. a crude extract—OeF.Cr). The yield was approximately 7%. OeF.Cr was completely solubilized in saline/distilled water for in vivo and in vitro use.

Animals and chemicals

Animals used in this study were housed at the Animal House of the Aga Khan University, maintained at 23–25°C and given a standard diet and tap water ad libitum. The experiments performed complied with the guidelines of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (1996) and were approved by the Ethical Review Committee of the Aga Khan University.

Acetylcholine perchlorate, atropine sulphate, (±)-isoproterenol hydrochloride, (±)-norepinephrine bitartrate, l-phenylephrine hydrochloride, potassium chloride and verapamil hydrochloride were purchased from Sigma Chemicals Co. (St Louis, MO, USA) and sodium thiopental was obtained from Abbot Laboratories, Karachi (Pakistan). All drugs were dissolved as stock solutions in distilled water and dilutions were made fresh in normal saline (0.9% sodium chloride) on the day of the experiment.

In-vivo experiments

In these experiments, Sprague–Dawley rats (180–200 g) were used and the arterial blood pressure was recorded as described previously (Gilani 1991). Rats were anaesthetized with an intraperitoneal injection of thiopental sodium (Pentothal, 70–90 mg/kg body weight) and the arterial blood pressure was recorded through carotid artery cannulation via a pressure transducer (P23 XL) coupled to a Grass model 7 Polygraph (Grass Instruments Company, MA, USA). Drugs were injected through a cannula inserted into the jugular vein. After a 20 min period of equilibrium, the rats were injected intravenously with 0.1 ml saline or the same volume of a test substance.

Arterial blood pressure was allowed to return to the resting level between injections. Changes in blood pressure were recognized as the difference between the steady state values before and the peak readings after injection. The mean blood pressure was calculated as the diastolic blood pressure plus one-third of the pulse width.

In-vitro experiments

To study the possible mechanism of action we used isolated tissue preparations. These experiments were carried out by methods employed previously in our laboratory (Gilani et al. 1994, 1999).

Guinea-pig atria

Guinea pigs (450–500 g) of either sex were killed by cervical dislocation. The atria were dissected out, cleaned of fatty tissue and mounted in 20 ml tissue baths containing Kreb's solution, maintained at 32°C and aerated with a mixture of 95% O_2 and 5% CO_2 (carbogen). The composition of Kreb's solution was: NaCl, 118.2 mM; $NaHCO_3$, 25.0 mM; $CaCl_2$, 2.5 mM; KCl, 4.7 mM; KH_2PO_4, 1.3 mM; $MgSO_4$, 1.2 mM; and glucose, 11.7 mM (pH 7.4). Right atria, due to the presence of pacemaker cells, contract naturally. The tissues were allowed to beat spontaneously under a resting tension of 1 g. An equilibrium period of 30 min was allowed before the application of any drug. Control responses of acetylcholine (1 μM) and isoproterenol (1 μM) were obtained at least in duplicate. Tension changes in the tissue were recorded via a Grass force-displacement transducer (model FT-03) using a Grass Model 7 Polygraph.

Rabbit aorta

Rabbits (1.0–1.2 kg) of either sex were sacrificed by a blow on the back of the head. The descending thoracic aorta was removed and cut into 2–3 mm wide rings, which were individually mounted in 20 ml tissue baths containing Kreb's solution, maintained at 37°C and bubbled with carbogen. A resting tension of 2 g was applied to each tissue and an equilibrium period of 1 h was allowed before studying the effect of test materials. Changes in isometric tensions of the rings were measured via a force-displacement transducer (FT-03), using a Grass model 7 Polygraph. Phenylephrine (1 μM) doses were used to stabilize the preparation.

Determination of calcium antagonist activity

To assess whether the blood pressure lowering action of the plant extract was through calcium channel blockade, High K^+ (80 mM) was added to the tissue bath, which produced a sustained contraction (Farre et al. 1991). Test material was then added in a cumulative fashion to obtain concentration-dependent inhibitory responses (Van Rossum 1963). Relaxation of aortic preparations, pre-contracted with K^+ (80 mM), was expressed as a percentage of the control responses mediated by K^+.

To confirm the calcium antagonist activity of test substances, the tissue was stabilized in normal Kreb's solution, then placed in Ca^{2+}-free Kreb's solution containing ethylenediamine tetraacetic acid (0.1 mM) for 30 min to remove calcium from the tissues. This solution was further replaced with K^+-rich and Ca^{2+}-free

Kreb's solution, having the following composition: KCl, 50 mM; NaCl, 50.58 mM; $MgSO_4$, 3.10 mM; $NaHCO_3$, 23.8 mM; KH_2PO_4, 1.26 mM; glucose, 11.1 mM; and ethylenediamine tetraacetic acid, 0.1 mM. Following an incubation period of 1 h, control dose–response curves of Ca^{2+} were obtained. When the control dose–response curves of Ca^{2+} were found super-imposable (usually after two cycles), the tissue was pretreated with the plant extract for 60 min to test the possible calcium channel blocking effect. The dose–response curves of Ca^{2+} were reconstructed in the presence of different concentrations of the test material.

Results and discussion

Intravenous administration of a saline solution of the aqueous–methanolic extract of *O. europea* fruit (OeF.Cr) caused a fall in systolic, diastolic and mean arterial blood pressures in normotensive anaesthetized rats. The effect of OeF.Cr was in a dose-dependent manner (30–100 mg/kg), with a maximum fall in mean arterial blood pressure of 37.12 ± 0.30% (mean ± standard error of the mean, $n = 6$), as shown in Figure 1. The figure shows a tracing from a typical experiment, whereas combined data from different experiments are presented in Figure 2. Figure 1 also compares plant extract and acetylcholine for their blood pressure lowering effects before and after treatment with atropine. Acetylcholine (1 μg/kg) produced a considerable drop in blood pressure qualitatively similar to that of plant extract. Pretreatment of animals with atropine (1 mg/kg), a competitive blocker of acetylcholine at muscarinic receptors (Arunlakshana & Schild 1959; Gilani et al. 1997), did not alter the hypotensive effect of plant extract. On the other hand, the acetylcholine response was completely abolished, suggesting that, unlike acetylcholine, plant extract mediates its

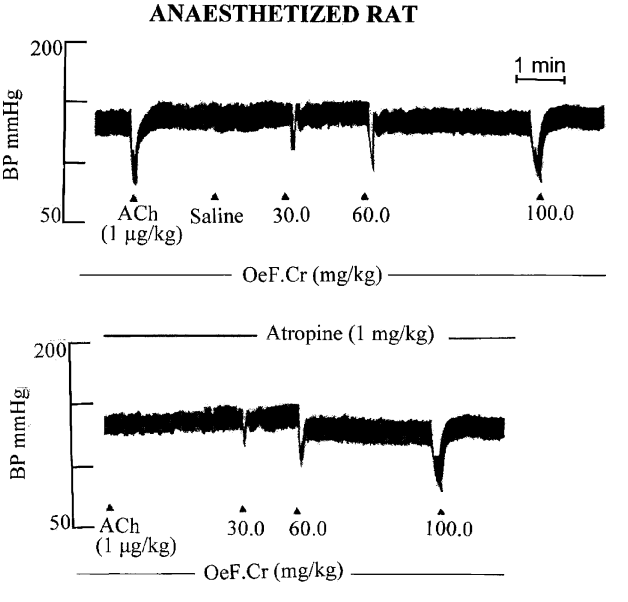

Figure 1. Comparison of the crude extract of *O. europea* fruit (OeF.Cr) and acetylcholine (ACh) for their effects on blood pressure (BP) in the absence and presence of atropine in an anaesthetized rat. Small triangles show the times at which the drugs were administered.

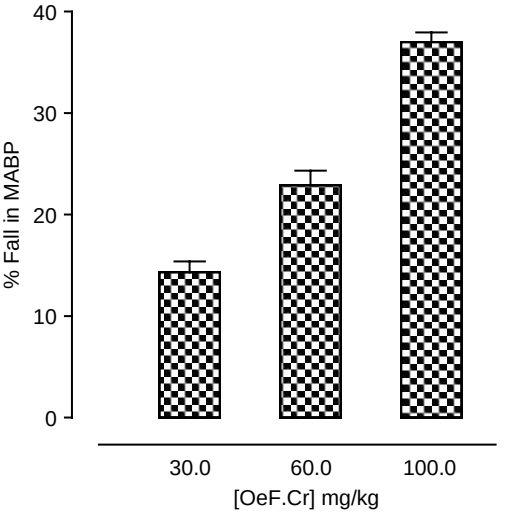

Figure 2. Dose-dependent effects of crude extract of *O. europea* fruit (OeF.Cr) on the mean arterial blood pressure (MABP) in anaesthetized rats. Values presented as mean ± standard error of the mean, $n = 6$.

blood pressure lowering action through a mechanism independent of muscarinic receptor activation.

It is customary to use isolated tissue preparations to explore possible mechanism of action, as response interference from intact reflex is obliterated. When tested on the spontaneously beating right atria, OeF.Cr suppressed the atrial force of contraction and rate in the dose range of 0.1–3.0 mg/ml (Figure 3). This property of plant extract may be partly responsible for the blood pressure lowering effect observed in the anesthetized rats, as blood pressure is the product of cardiac output and peripheral resistance (Johansen 1992). The cardiac inhibitory effect of the plant extract was

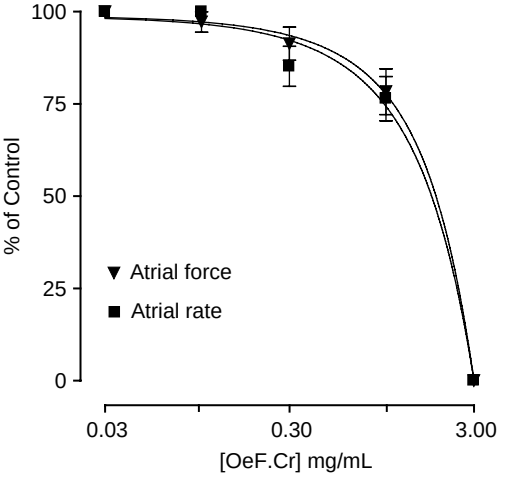

Figure 3. Inhibitory effect of the crude extract of *O. europea* fruit (OeF.Cr) on the force of contraction and rate of spontaneously beating isolated guinea-pig right atria preparations. The symbols represent mean ± standard error of the mean, $n = 4$.

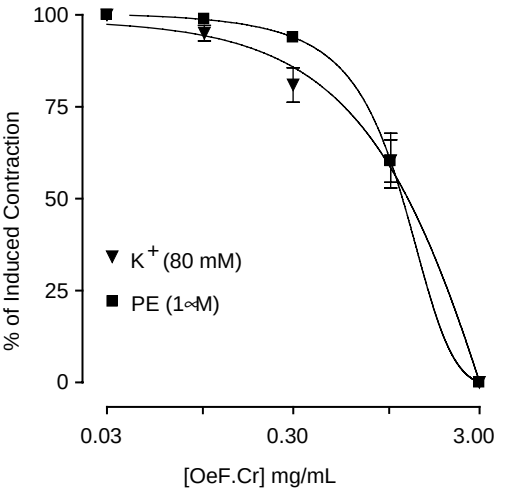

Figure 4. Dose-dependent inhibitory effect of the crude extract of *O. europea* fruit (OeF.Cr) on K^+ and phenylepherine (PE)-induced contractions in isolated rabbit aorta preparations. The symbols represent mean ± standard error of the mean, $n = 4$.

atropine resistant, indicating that the plant extract does not cause cardiac relaxation by a mechanism similar to that of acetylcholine.

When tested on vascular preparations at resting tension, the plant extract was found devoid of any vasoconstrictor effect (data not shown). However, OeF.Cr caused dose-dependent (0.1–3.0 mg/ml) relaxation of aortic rings pre-contracted with K^+ (80 mM) or phenylepherine (1 μM) as shown in Figure 4. At high doses (>30 mM), K^+ is known to markedly contract blood vessels by depolarizing smooth muscle cells and increasing influx of Ca^{2+} through L-type voltage-sensitive calcium channels (Bolton 1979; Godfraind et al. 1986). Contractions induced by phenylepherine (an α_1-receptor agonist) and adrenergic neurotransmitters are partly due to influx of extracellular Ca^{2+} via receptor-operated calcium channels and partly due to Ca^{2+} release from intercellular stores (Graham et al. 1996). Inhibition by the crude extract of both K^+ and phenylepherine-induced contractions was observed at a similar concentration range, suggesting that a non-specific vasodilator action, possibly mediated through calcium antagonism, may be responsible for blood pressure lowering effect in anaesthetized rats.

The existence of calcium antagonistic activity was confirmed when the plant extract caused a dose-dependent rightward shift in the Ca^{2+} dose–response curves, similar to verapamil, a standard calcium channel blocker (Fleckenstein 1977) as shown in Figure 5. Calcium antagonists are therapeutically important in various cardiovascular disorders, such as hypertension (Triggle 1992). The common characteristic of these drugs is their dose-dependent inhibition of the slow entry of Ca^{2+} and a capacity for the reversal of this effect by calcium ions (Fleckenstein 1977). The observed effect of the plant extract to inhibit the induced contractions, followed by displacing the Ca^{2+} curves similar to verapamil, strongly suggests the calcium antagonistic activity of olive extract. These results clearly indicate that the blood pressure lowering effect of olives is possibly mediated through calcium channel blockade, and this also explains its traditional use in hypertension.

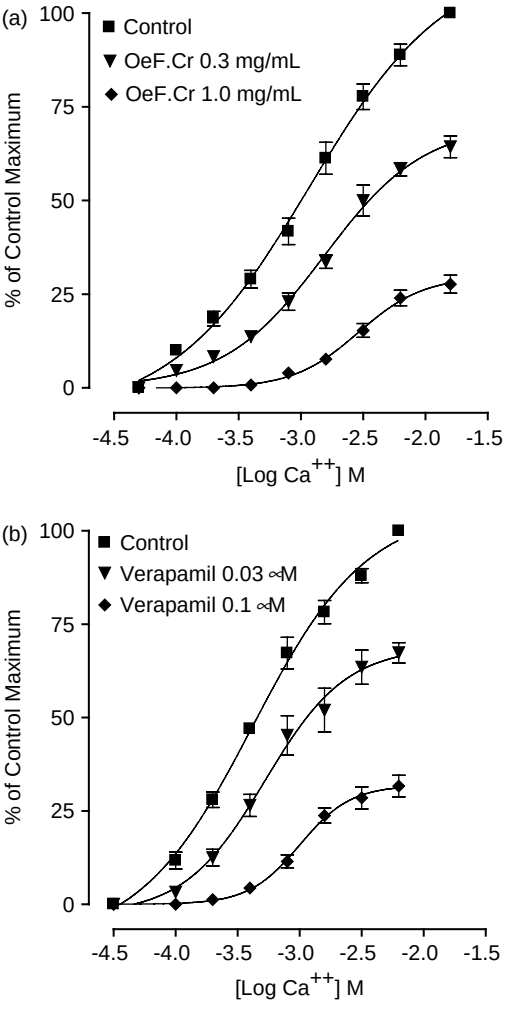

Figure 5. Dose–response curves of Ca^{2+} in the absence and presence of increasing doses of (a) crude extract of *O. europea* fruit (OeF.Cr) and (b) verapamil in isolated rabbit aorta preparations. The symbols represent mean ± standard error of the mean, $n = 3–4$.

Acknowledgements

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