AIRWAYS AND CARDIOVASCULAR INHIBITORY EFFECTS OF *OLEA EUROPEA* AND *TERMINALIA BELLERICA* AQUEOUS FRACTIONS

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Abstract

This study describes the bronchodilatory, vaso-relaxant and cardiac inhibitory effects of aqueous fractions of *Olea europea* (Oe.Aq) and *Terminalia bellerica* (Tb.Aq) fruits. In guinea-pig tracheal preparations, Oe.Aq and Tb.Aq caused concentration-dependent (0.03-5 mg/mL) relaxation of carbachol (CCh, 1 µM) and high potassium (80 mM)-induced contractions. When tested on rabbit aortic rings, against phenylephrine (PE, 1 µM) and potassium (K^+)-induced contractions, Oe.Aq and Tb.Aq caused non-specific inhibition of the induced contractions. In isolated guinea-pig atria, Oe.Aq and Tb.Aq exhibited a suppressive effect on the atrial force and the rate of contractions. These data indicate that *Olea europea* and *Terminalia bellerica* aqueous fractions possess airways and cardiovascular inhibitory activities, via calcium antagonism like verapamil.

Rezumat

Studiul experimental prezintă efectul bronhodilatator, vasorelaxant şi inhibitor cardiac al extractelor apoase obţinute din fructele speciilor *Olea europea* şi *Terminalia bellerica.*

Rezultatele obţinute au demonstrat acţiunea bronhodilatatoare şi inhibitorie la nivel cardiovascular, printr-un mecanism ce presupune antagonismul cu ionii de calciu, similar verapamilului.

Keywords: *Olea europea*; *Terminalia bellerica;* bronchodilator; vasodilatation; cardiac inhibitory effect

Introduction

Olea europea (olive) is a small-growing evergreen tree native in the southern Europe and Asia Minor. The plant has a folkloric reputation as an aphrodisiac, emollient, laxative, nutritive, resolvent, sedative, tonic and is also considered useful to treat abdominal spasm [9] and hypertension [10]. *Terminalia bellerica* Roxb. (*Combretaceae*) commonly known as "belleric myrobalan" is a large deciduous tree, found throughout Central Asia and some other parts of the world [6]. Its fruit is used in folk medicine to treat anemia, asthma, cancer, spasm, cough, diarrhea, dyspepsia, dysuria, headache, hypertension, inflammations and rheumatism [1].

Materials and methods

Chemicals and animals

Acetylcholine chloride, carbachol (CCh), isoprenaline hydrochloride, phenylephrine hydrochloride (PE), potassium chloride and verapamil were purchased from Sigma Chemicals Co, St Louis, MO, USA. The animals used in this study were thirteen adult rabbits (1-1.5 kg) and twelve guinea-pigs (450-500 g) of either sex and were housed at Animal House of Aga Khan University. Experiments performed complied with the rules of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council [8] and were approved by the Ethical Committee of Aga Khan University.

Preparation of plant aqueous fractions

The *Olea europea* and *Terminalia bellerica* fruits were sliced and soaked individually in 70% aqueous-methanol solution. The initial preparation was filtered through a muslin cloth, then through Whatman filter paper. This procedure was repeated twice and the combined filtrate was evaporated using a rotary evaporator under reduced pressure. About 15 g of *Olea europea* and 10 g of *Terminalia bellerica* hydro-methanolic material were individually dissolved in about 200 mL of distilled water and after the treatment with different organic solvents: petroleum spirit, chloroform and ethyl acetate (separation performed using the separating funnel), the final lower layer was concentrated to obtain the aqueous fractions of *Olea europea* (Oe.Aq) and *Terminalia bellerica* (Tb.Aq), yielding 73.3% and 61.5% respectively.

Guinea-pig trachea

Nine guinea-pig tracheas obtained from animals, sacrificed by cervical dislocation, were dissected and kept in Krebs solution [4]. The preparation was then mounted in 20 mL tissue baths containing Kreb's solution, maintained at 37º C and aerated with carbogen. A tension of 1 g was applied to each of the tracheal strip and was kept constant throughout the experiment. Changes in isometric tensions of strips were measured via force-displacement transducer (FT-03) using Grass model 7 Polygraph (Grass Instrument Company, Quincy, MA, USA).

Rabbit aorta

Thirteen thoracic aortas from rabbits, killed by blow on back of the head were isolated. Aortic rings of 2-3 mm width were mounted in 20 mL tissue baths containing Krebs solution, at 37ºC aerated with carbogen. A resting tension of 2 g was applied to each tissue [7]. The changes in isometric tensions of rings were measured using Grass model 7 Polygraph.

Guinea-pig atria

Twelve right atrias isolated from guinea-pigs were mounted in 20 mL tissue baths containing Krebs solution, at 32ºC and supplied carbogen

[4]. Tissues were allowed to beat spontaneously (due to the presence of pacemaker cells) under the resting tension of 1 g. Tension changes were recorded using the Grass model 7 Polygraph.

Statistical analysis

Data are expressed as mean ± standard error of mean (SEM, n=number of determinations) and the median effective concentrations (EC_{50}) with 95% confidence intervals (CI).

Results and discussion

In tracheal preparations, Oe.Aq, Tb.Aq, and verapamil caused concentration-dependent inhibition of the CCh (1 μM) and potassium (K^+, 80 mM)-induced contractions with respective EC_{50} values of 2.2 mg/mL (1.1-4.8 mg/mL, 95% CI, n=3) and 2.1 mg/mL (1.02-4.2 mg/mL, n=3), 3.16 mg/mL (3.1-3.3 mg/mL, n=3) and 3.21 mg/mL (1.9-5.5 mg/mL, n=3), 0.26 μM (0.17-0.33 μM, n=3) and 0.05 μM (0.03-0.06 μM, n=3) as shown in Figure 1. Oe.Aq, Tb.Aq, and verapamil caused the relaxation of phenylephrine (PE, 1 μM) and K^+ (80 mM)-induced contractions of aortic rings with EC_{50} values of 1.6 mg/mL (1.0-2.7 mg/mL, n=5) and 1.7 mg/mL (1.0-2.9 mg/mL, n=5), 1.4 mg/mL (0.84-2.3 mg/mL, n=3) and 1.7 mg/mL (1.0-2.9 mg/mL, n=3), 1.42 μM (1.0-2.0 μM, n=5) and 1.0 μM (0.80-1.3 μM, n=5) respectively (Figure 2). Oe.Aq, Tb.Aq, and verapamil caused concentration-dependent suppression of the force and rate of atrial spontaneous contractions, with EC_{50} values of 1.8 mg/mL (1.0-3.1 mg/mL, n=4) and 1.4 mg/mL (0.81-2.4 mg/mL, n=4), 2.6 mg/mL (1.4-3.0 mg/mL, n=4) and 3.3 mg/mL (2.1-4.9 mg/mL, n=4), 0.90 μM (0.70-1.2 μM, n=4) and 1.1 μM (0.82-1.6 μM, n=3) respectively (Figure 3).

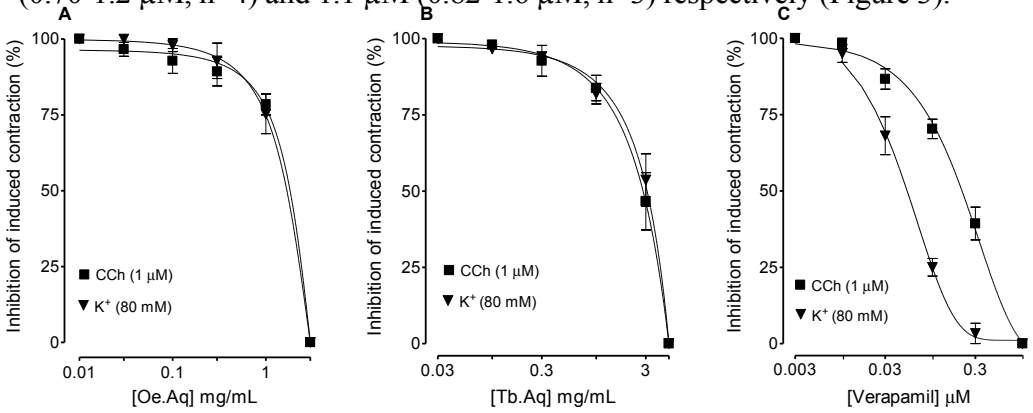

Figure 1

Concentration-dependent inhibitory effect of **(A)** *Olea europea* aqueous fraction (Oe.Aq), **(B)** *Terminalia bellerica* aqueous fraction (Tb.Aq) and **(C)** verapamil on the carbachol (CCh) and high K^+ induced contractions of isolated guinea-pig tracheal preparations. Values shown are mean ± SEM, n=3.

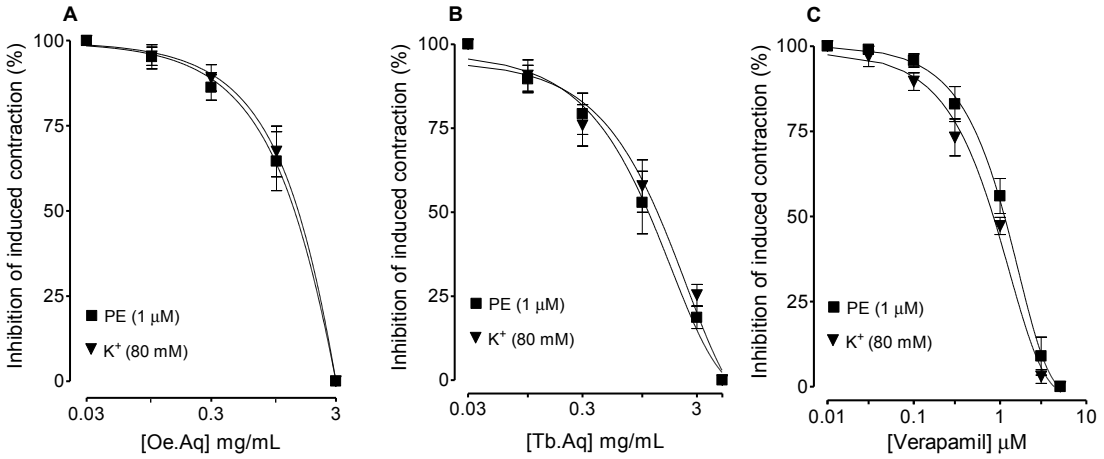

Figure 2

Inhibitory effect of **(A)** *Olea europea* aqueous fraction (Oe.Aq), **(B)** *Terminalia bellerica* aqueous fraction (Tb.Aq) and **(C)** verapamil on phenylephrine (PE) and high K^+ induced contractions of isolated rabbit aortic preparations. Values shown are mean ± SEM, n=3-5

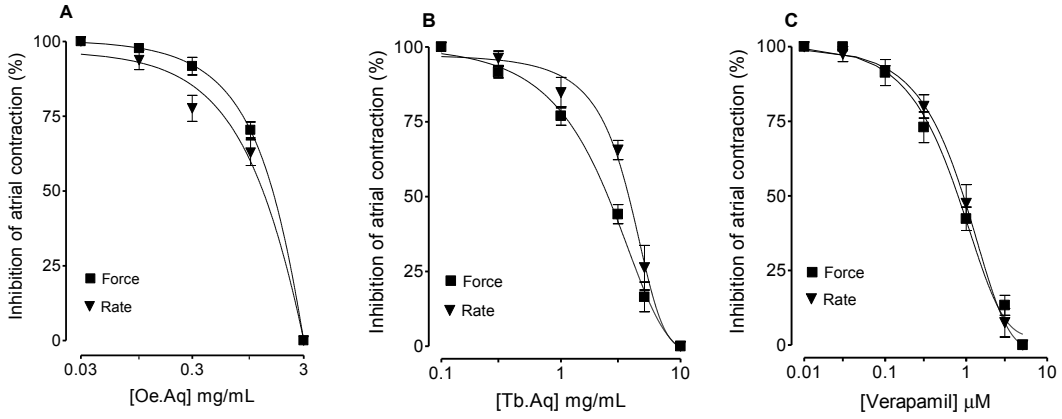

Figure 3

Concentration-dependent inhibitory effect of **(A)** *Olea europea* aqueous fraction (Oe.Aq), **(B)** *Terminalia bellerica* aqueous fraction (Tb.Aq) and **(C)** verapamil on the force and rate of isolated guinea-pig right atrial preparations. Values shown are mean ± SEM, n=3-4

When tested on guinea-pig trachea, the *Olea europea* and *Terminalia bellerica* aqueous fractions relaxed both the CCh and high K^+-induced contractions, like verapamil, a standard Ca^{++} antagonist [3]. High levels of K^+ (> 30 mM) and CCh, a cholinergic agonist, are known to cause smooth muscle contractions through opening voltage-dependent Ca^{++} channels and stimulation of muscarinic receptors respectively, eventually leading to increased intracellular Ca^{++} levels [4], resulting in a bronchoconstrictor effect. Inhibitory effects of the vegetal extracts against

the two spasmogens, indicate their non-specific bronchodilatory action, as exhibited by verapamil.

Oe.Aq and Tb.Aq were studied further for the possible vaso-relaxant and cardio-suppressant effects. In rabbit aortic rings, both plant fractions caused an inhibition of the PE and high K^+-induced contractions, similar to that observed in the case of verapamil. PE produces vascular contraction through an increase in cytosolic Ca^{++}, partly due to Ca^{++} influx via receptor operated channels and partly via Ca^{++} release from intracellular stores [5]. The inhibition of both PE and K^+-induced contractions by Oe.Aq and Tb.Aq reveals their vasodilatory effect, via Ca^{++} antagonist pathway.

When tested in spontaneously beating guinea-pig atria, Oe.Aq and Tb.Aq exhibited negative inotropic and chronotropic effects, similar to that caused by verapamil. The Ca^{++} antagonists are known to cause cardiac depression through inhibiting the slow inward current during the action potential plateau, leading to a decrease in the cardiac output [2] and thus falling blood pressure.

Conclusions

These results clearly indicate that *Olea europea* and *Terminalia bellerica* aqueous fractions possess bronchodilator and cardiovascular inhibitory effects, through a Ca^{++} antagonistic mechanism and thus provide an evidence for the therapeutic potential of both plants in respiratory and cardiovascular disorders.

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