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Cardiovascular inhibitory properties of *Lens culinaris*

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Abstract

In present study, we report the blood pressure lowering, vasodilatory and cardio-depressant activities of *Lens culinaris*. The crude extract of *L. culinaris* induced dose-dependent (3-30 mg/kg) fall in the arterial pressure of rats under anesthesia. When tested in rat aortic ring preparations, *L. culinaris* at concentration range of 0.03-5.0 mg/mL relaxed high K^+ (80 mM) and phenylephrine (1 µM)-induced contractions, like that caused by verapamil. In isolated guinea-pig atria, *L. culinaris* caused inhibition of atrial force and rate of spontaneous contractions, similar to that exhibited by verapamil. These data indicate that *L. culinaris* exhibits blood pressure lowering potential, mediated possibly through Ca^{++} channel blockade mechanism.

Introduction

We recently observed that *Lens culinaris* Moench (Leguminosae) exhibited gut and airways relaxant activities (Khan et al., 2014). In the current era, lot research is going on for exploration of alternative therapy for hypertension management (Ghayur and Janssen, 2010) because they are safe, economical and possesses synergistic and side-effect neutralizing properties (Gilani and Atta-ur-Rahman, 2005). In the present study, we evaluated the effects of *L. culinaris* on cardiovascular parameters and reported that it exhibits blood pressure lowering, cardio-depressant and vasodilator activities, mediated possibly via inhibition of Ca^{++} entry via membranous Ca^{++} channels.

Material and Methods

Plant material and extraction

The seeds were cleaned and approximately 1 kg of the material was soaked in the aqueous-methanol. All the filtrates were combined and evaporated to dryness on a Rotary Evaporator under reduced pressure (-760 mm Hg) at 35-40°C to a thick and dark brown mass, the crude extract of *Lens culinaris* (Lc.Cr), yielding approximately 3.6%.

Animals

Experimentsl animals used in this study include adult Sprague-Dawley rats (180-200 g) and guinea-pigs (450-500 g) of local breed and either sex housed in the animal facility of The Aga Khan University under controlled environment (23-25°C).

Chemicals

Acetylcholine chloride (ACh), isoprenaline hydrochloride, norepinephrine hydrochloride (NE), phenylephrine hydrochloride and verapamil hydrochloride were purchased from Sigma Chemical Company, USA. Pentothal sodium (thiopental) was obtained from Abbot Laboratories, Karachi, Pakistan.

Measurement of blood pressure

Rats were anaesthetized with thiopental sodium (Pentothal®, 70-90 mg/kg, i.p.) Trachea was cannulated with a

polyethylene tubing Pe-20 to maintain the spontaneous respiration (Consolini et al., 1999; Gilani et al., 2005). The right jugular vein was cannulated with polyethylene tubing Pe-50 to facilitate the intravenous (i.v) administration of different drugs. The left carotid artery was cannulated with similar tubing filled with heparinized saline 60 IU/mL and connected to a pressure transducer (MLT 0380/D Reusable BP-Transducer) coupled to ML 224 Quad Bridge Amplifier and PowerLab ML 4/25 data acquisition system (AD Instruments, Australia) for blood pressure recording. Following 20 min period of equilibrium, rats were injected intravenously with the test substance. Arterial blood pressure was allowed to return to resting level between injections. Mean arterial pressure (MAP) was calculated as the diastolic BP plus one-third of the pulse width (systolic blood pressure - diastolic blood pressure).

Rat aortic tissues

Rats were sacrificed by cervical dislocation. After abdominal opening, the thoracic aorta was dissected out, cleaned of fat and adipose tissues and cut into 3-5 mm long rings and mounted individually in a 5 mL tissue bath containing Kreb's solution. Resting tension of 1 g was applied to each tissue and an equilibrium period of 30 min was allowed before any experimentation. The tissues were then stabilized with repeated exposure (usually 3 times) to high KCl solution (Gilani et al., 2006; Khan et al., 2012). The test drug was applied for its ability to relax the contractions induced with high K^+ (80 mM) and phenylephrine (1 µM). The ability of extract to relax K^+ (80 mM)-induced contractions would indicate L-type voltage-operated calcium channel blocking (CCB) mode of vasodilation, while inhibition of the phenylephrine-induced contractions, would suggest the blockade of the Ca^{++} influx through receptor-operated Ca^{++} channels (Godfraind et al., 1986; Wang et al., 2001). Changes in tension were recorded and analyzed isometrically through a force transducer (Fort-10, WPI, UK) coupled to a bridge amplifier (Transbridge TBM 4M, WPI) and PowerLab ML 845 data acquisition system (AD Instruments, Australia).

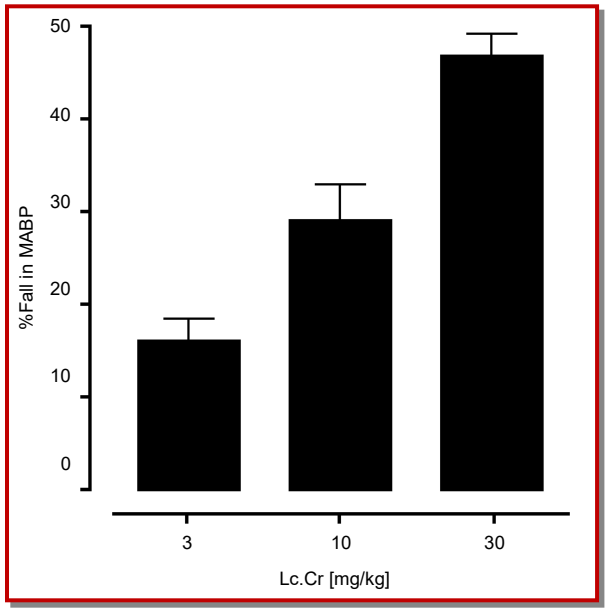

Figure 1: Dose-dependent hypotensive effect of *Lens culinaris* crude extract (Lc.Cr) on mean arterial blood pressure (MABP) in anesthetized rats. Values shown represent mean ± SEM, n = 3

Guinea-pig atrial preparations

In the *in vitro* assays, right atria isolated from the guinea pigs were mounted in 20 mL tissue baths containing Kreb's solution, at 32°C and aerated with carbogen. Each tissue was allowed to beat spontaneously (due to pacemaker) under the resting tension of 1 g (Khan and Gilani, 2008; Khan and Gilani, 2011; Khan et al., 2012). An equilibrium period of 45 min was allowed before the application of any drug. The drug-induced changes in force of atrial contractions were measured as the percent change in base-line values. Tension changes in the tissue were recorded via force-displacement transducer (FT-03) using Grass Model 7 Polygraph.

Results

Intravenous administration of *L. culinaris* caused dose-dependent fall of arterial pressure in the anaesthetized rats. The percent fall of pressure at 3, 10 and 30 mg/kg doses was 16.0 ± 2.3, 29 ± 3.7 and 46.6 ± 2.4% (n=3) respectively, as shown in Figure 1. When tested against high K^+ (80 mM) and phenylephrine (1 M)-induced contractions in isolated rat aortic ring preparations, *L. culinaris* exhibited a vasodilator effect with respective EC_{50} values of 0.28 mg/mL (0.2-0.4, n = 3) and 2.7 mg/mL (1.0-7.7, n=3) thus showing more potency against high K^+ as shown in Figure 2A. Similarly, verapamil inhibited the high K^+ (80 mM) and PE (1 M)-induced contractions by showing higher potency against K^+ with EC_{50} values of 0.3 µM (0.2-0.4, n=3) and 1.0 µM (0.8 -1.3, n=3) respectively (Figure 2B). In isolated guinea-pig atria, *L. culinaris* exhibited concentration-dependent inhibitory effect on force and rate of spontaneous contractions (Figure 3A) with respective EC_{50} values of 5.6 (5.1-6.0, 95% CI, n=3) and 6.3 mg/mL (5.4-6.7, n=3). Similarly, verapamil caused concentration-dependent inhibitory effect with EC_{50} values of 0.8 (0.6-0.9, n=3) and 1.0 M (0.8-1.1, n=3) respectively (Figure 3B).

Discussion

The aqueous-methanolic *L. culinaris* extract in dose-dependent fashion caused fall in the arterial BP of rats under anesthesia. As the blood pressure is considered the product of cardiac output and peripheral vascular resistance (Johansen, 1992), the plant extract was

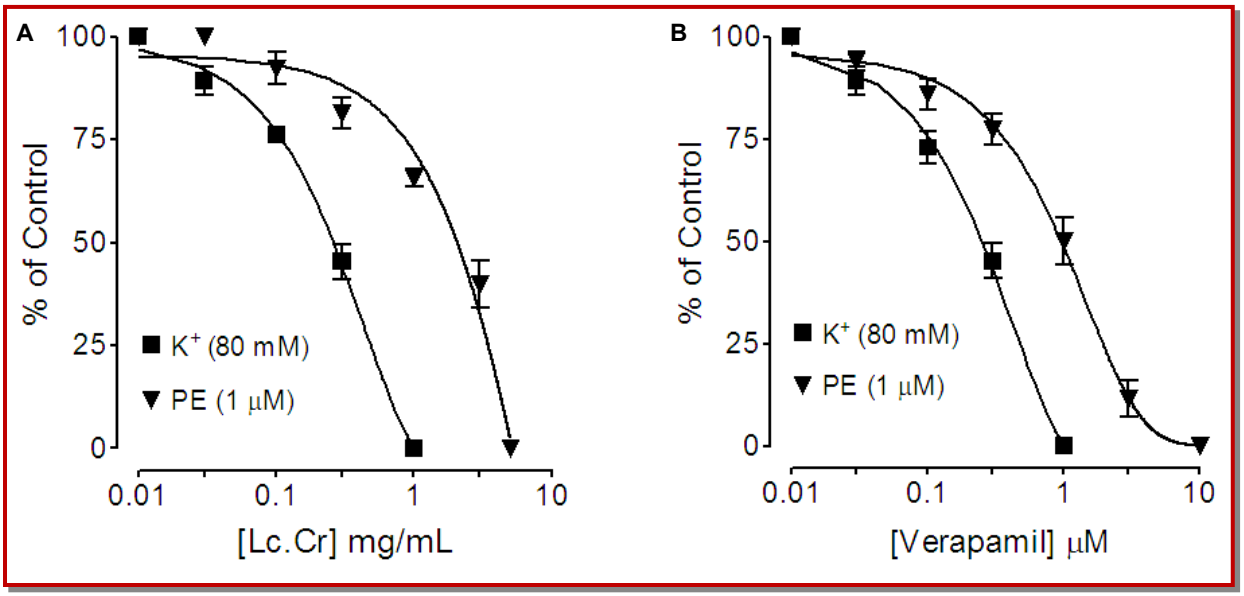

Figure 2: Concentration-dependent relaxant effects of (A) *L.culinaris* crude extract (Lc.Cr) and (B) verapamil on high K^+ and phenylephrine (PE)-induced contractions in isolated rat aortic ring preparations. Values shown are mean ± SEM, n = 3

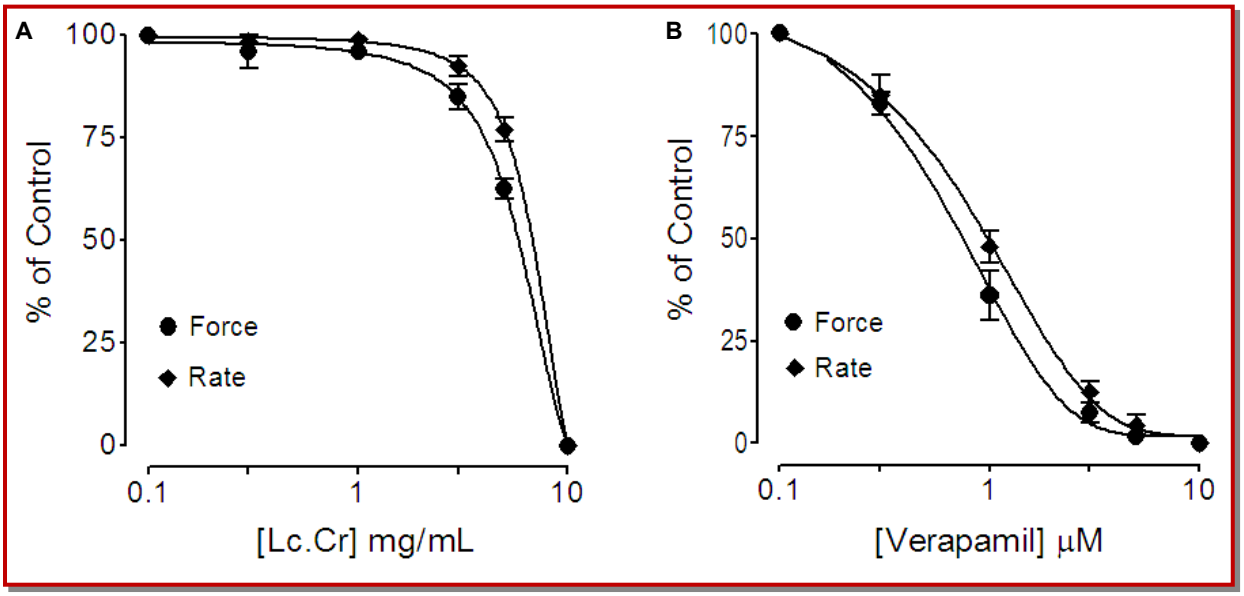

Figure 3: Concentration-response curves showing the inhibitory effect of (A) *L.culinaris* crude extract (Lc.Cr) and (B) verapamil on force and rate of spontaneous contractions in isolated guinea-pig right atria. Values shown are mean ± SEM, n = 3

studied in heart and vascular preparations for the possible cardio-depressant and vasodilator actions. To see effect on vascular resistance, *L. culinaris* was studied in rat thoracic aorta, which is routinely used for elucidating underlying mechanism(s) of blood pressure -lowering effect (Ghayur and Gilani, 2005; Ajay et al., 2007). In isolated rat aortic tissues, *L. culinaris* extract was screened against high K^+ and phenylephrine-induced contractions. *L. culinaris* relaxed high K^+ and phenylephrine-induced contractions in aortic rings, indicating that it was acting through blockade of voltage- and receptor-operated Ca^{++} channels (Okmura et al., 1993; Musha et al., 2005; Khan et al., 2013). As *L. culinaris* was found relatively more potent against K^+ (having 10 times lower EC_{50}) as compared to phenylephrine-induced contractions, thus showing greater potency to block the voltage-sensitive Ca^{++} channels as comparison to the receptor-operated Ca^{++} channels, a pattern of activity similar to verapamil, a reference Ca^{++} antagonist (Fleckenstein, 1977). *L. culinaris* was more potent in its inhibitory effect on vascular tissues than cardiac. There is sufficient evidence of heterogeneity of Ca^{++} channels and different Ca^{++} antagonists exhibit selectivity for different organ systems (Farre et al., 1991). For example, dihydropyridine antagonists are considered selective for vascular tissues and are more

commonly used to lower blood pressure (Joseph and Barry, 1999).

In guinea-pig atria, *L. culinaris* exhibited negative inotropic and chronotropic effects. The cardiac inhibitory action of the *L. culinaris* may be due to Ca^{++} antagonist effect leading to decrease in cardiac output and thus falling blood pressure.

Conclusion

L. culinaris exhibits blood pressure-lowering, vasodilatory and cardio-depressant effects mediated possibly Ca^{++} channel blockade pathway.

References

Ajay M, Chai HJ, Mustafa AM, Gilani AH, Mustafa MR. Mechanisms of the anti-hypertensive effect of *Hibiscus sabdariffa* L. calyces. J Ethnopharmacol. 2007; 109: 388-93.

Consolini AE, Baldini OAN, Amat AG. Pharmacological basis for the empirical use of *Eugenia uniflora* L. (Myrtaceae) as antihypertensive. J Ethnopharmacol. 1999; 66: 33-39.

Farre AJ, Columbo M, Fort M, Gutierrez B. Differential effects of various Ca^{++} antagonists. Gen Pharmacol. 1991; 22: 177-81.

Fleckenstein A. Specific pharmacology of Ca^{++} in myocardium, cardiac pacemakers and vascular smooth muscle. Rev Pharmacol Toxicol. 1977; 17: 149-66.

Ghayur MN, Gilani AH. Ginger lowers blood pressure through blockade of voltage-dependent calcium channels. J Cardiovasc Pharmacol. 2005; 45: 74-80.

Ghayur MN, Janssen LJ. A natural way to cardiovascular health. Nat Rev Cardiol. 2010; 7: 1-2.

Gilani AH, Atta-ur-Rahman. Trends in ethnopharmacology. J Ethnopharmacol. 2005; 100: 43-49.

Gilani AH, Ghayur MN, Houghton PJ, Jabeen Q, Kazim SF, Jumani MI, Saeed SA. Studies on the hypotensive, cardio-suppressant, vasodilator and antiplatelet activities of betel nut crude extract and its constituents. Int J Pharmacol. 2006; 2: 33-41.

Gilani AH, Khan AU, Shah AJ, Cornor JD, Jabeen Q. Blood pressure lowering effect of olive is mediated through calcium channel blockade. Int J Food Sci Nutrit. 2005; 56: 613 -20 .

Godfraind T, Miller R, Wibo M. Calcium antagonism and calcium entry blockade. Pharmacol Rev. 1986; 38: 321-416.

Johansen PL. Hemodynamic effects of calcium antagonists in hypertension. In: Calcium antagonists in clinical medicine. Epstein M, (ed). Philadelphia USA. Hanley and Belfus. 1992, pp 62-98.

Joseph JS, Barry LC, Hypertension In: DiPiro, JT, Talbert RL, Yee GC, Matzke GR, Wells BG, Posey LM, (eds). The pharmacotherapy: A pathophysiologic approach. 6th ed. New York, McGraw-Hill, 1999, p 208.

Khan A, Gilani AH. Pharmacodynamic evaluation of *Terminaliabellerica* for its antihypertensive effect. J Food Drug Anal. 2008; 16: 6-14.

Khan AU, Gilani AH . Blood pressure lowering, cardiovascular inhibitory and bronchodilatory activities of *Achillea millefolium*. Phytother Res. 2011; 25: 577-83.

Khan M, Khan AU, Najeeb-ur-Rehman, Gilani AH. Pharmacological basis for the medicinal use of *Lens culinaris* in gastrointestinal and respiratory disorders. Phytother Res. 2014; doi: 10.1002/ptr.5136: [Epub ahead of print] .

Khan M, Khan AU, Najeeb-ur-Rehman, Zafar MA, Hazrat A, Gilani AH. Cardiovascular effects of *Juniperus excelsa* are mediated through multiple pathways clinical and experimental hypertension. Clin Exp Hypertens. 2012; 34: 209-16.

Khan M, Shah AJ, Gilani AH. Antidiarrheal and antispasmodic activities of Vitex negundo are mediated through calcium channel blockade. Bangladesh J Pharmacol. 2013; 8: 317-22.

Musha S, Watanabe M, Ishida Y, Kato S, Konishi M, Tomoda A. A phenoxazine compound, 2-amino-4,4α-dihydro-4α-7-dimethyl-3H-phenoxazine-3-one reverses the phenylephrine or high-K^+ induced contractions of smooth muscles in rat aorta and guinea-pig tenia cecum. Biol Pharm Bull. 2005; 28: 1521-23.

Okmura K, Ichihara K, Nagaska M, Oda N, Tajima K. Calcium entry blocking activities of MPC-1304 and of its enantiomers and metabolites. Eur J Pharmacol. 1993; 235: 69-74.

Wang GJ, Shum AY, Lin YL, Liao JF, Wu XC, Ren J, Chen CF. Calcium channel blockade in vascular smooth muscle cells: major hypotensive mechanism of S-petasin, a hypotensive sesquiterpene from *Petasites formosanus*. J Pharmacol Exp Ther. 2001; 297: 240-46.

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