Arch. Pharm. Res. Vol. 17, No. 3, pp. 150-153, 1994

Cardiovascular Actions of *Daucus carota*

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(Received June 11, 1993)

Ethanolic extract of *Daucus carota* (*DC*) at the dose of 10-100 mg/kg caused a dose-dependent fall in systolic and diastolic arterial blood pressure in normotensive anesthetized rats. These effects were not blocked by atropine (1 mg/kg) and pretreatment with *DC* did not alter the pressor response to norepinephrine indicating that cardiovascular effects of *DC* are independent of cholinergic or adrenergic receptors involvement. In spontaneously beating guinea-pig paired atria, *DC* induced a concentration-dependent (0.3-5 mg/ml) decrease in force and rate of atrial contractions. In rabbit thoracic aorta, *DC* caused inhibition of K^+-induced contractions at similar concentrations. These results suggest that the extract may exhibit Ca^{2+} channel blocking-like direct relaxant action on cardiac and smooth muscle preparations and this action may be responsible for its hypotensive effect observed in the *in vivo* studies.

Key words: *Daucus carota*, Hypotensive, Spasmolytic, *In vivo*, *In vitro*

INTRODUCTION

Daucus carota Linn. (carrot) belongs to family Umbelliferae and is abundantly cultivated all over Pakisitan and India (Nadkarni, 1976; Murray, 1989). Different parts of plants have been used in the indigenous system of medicine; seeds are used as nerve tonic, and carminative while fruits are recommended in chronic diarrhoea, dropsy and also used as blood purifier (Nadkarni, 1976). Seeds are also used as aphrodisiac (Said, 1972) and in uterine pains (Murray, 1989). The plant has undergone extensive phytochemical studies and a large number of pure compounds have been isolated (Duke, 1992), however, it has not been widely studied for pharmacological actions. Rencently, seed extract of *Daucus carota* was shown to possess abortifacient effect in rats (Kaliwal *et al.*, 1984). Similarly, seed extract was reported to possess hypotensive and spasmolytic activities in an old study (Agarwal *et al.*, 1953), in which, detailed investigation on mechanism of action was not carried out. Also, pharmacological studies on carrot leaves have not been widely carried out, particularly for its effects on blood pressure. In the present investigation, we have studied hypotensive action of ethanolic extract of *Daucus carota* leaves in anesthetized rats and explored mechanism of action using isolated tissue preparations.

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MATERIALS AND METHODS

Plant Material

The fresh and uncrushed aerial parts of *Daucus carota* plant were collected from Thatta region (Sindh), Pakistan in 1991 and identified with the help of a botanist at the University of Karachi. The plant material (25 Kg) was soaked in 80% ethanol and kept at room temperature for one week. This procedure was repeated twice and the combined ethanolic extract was evaporated to dryness on a rotary evaporator and a dark greenish brown material was obtained, yielding approximately 8%. The extract was dissolved in saline for the assessment of biological activity.

Drugs

Drugs were obtained from the following sources: acetylcholine chloride, atropine sulphate, norepinephrine hydrochloride and phentolamine hydrochloride (Sigma Chem. Co. St. Louis, MO, USA) and thiopental sodium (Abbott Laboratories, Karachi, Pakistan). Stock solutions of all drugs were made in distilled water and

diluted with freshly made normal saline on the day of experiments.

PHARMACODYNAMIC STUDIES WITH ANESTHETIZED RATS

In these experiments, adult male Sprague-Dawley rats (200-250 g) were used and arterial blood pressure was recorded as described previously (Gilani, 1991). The animals were anesthetized with an intraperitoneal injection of sodium thiopentone (Pentothal, 70-90 mg/kg body weight). The right carotid artery was cannulated with heparinized polyethylene tubing PE-50, which was connected to a pressure transducer (Statham P23 AC) coupled with (a Grass model 79 D) polygraph. This connection was used for blood pressure recording. Heart rate was measured from the lead II ECG by using a Grass tachograph (model 7 PAF).

The left jugular vein was cannulated with similar tubing to facilitate the intravenous injection of the drugs and plant material. The exposed surface with the cannulation was covered with cotton wool moistened in warm saline. The rats were injected with heparin (1000 u/kg body weight) to prevent blood clotting.

After a 20 minute period of equilibrium, the rats were injected intravenously with 0.2 ml saline or with the same volume of test substances. Arterial blood pressure was allowed to return to the resting level between injections. Changes in blood pressure and heart rate were recognized as the difference between the steady state values before and the peak readings after injection. Mean blood pressure was calculated as the diastolic blood pressure plus one-third pulse width.

Isolated Tissue Experiments

These experiments were carried out by the method previously described (Gilani, 1989, 1991).

Guinea-pig Atria: Guinea-pigs of either sex (400-600 g) were killed by cervical dislocation. Paired atria were removed carefully and mounted into a 20 ml tissue bath filled with Krebs-Henseleit solution maintained at 35°C and aerated with 5% carbon dioxide in oxygen. The composition of the physiological salt solution was (mM): NaCl, 118.2; KCl, 4.7; $MgSO_4$, 1.3; KH_2PO_4, 1.2; D-glucose, 11.7; $NaHCO_3$, 25.0 and $CaCl_2$, 2.5. The spontaneous atrial contractions were recorded via a force-displacement transducer (FT-03) together with rate of contractions which were monitored with a Grass model 7 PAF tachograph. All experimental records were made on a Grass model 79 D polygraph. The preparation was allowed to equilibrate under 1 g resting tension for at least 30 min before administration of any drug. The drug-induced changes in the force and rate of atrial contractions were measured as the percent change in base line values obtained immediately before addition of a test substance.

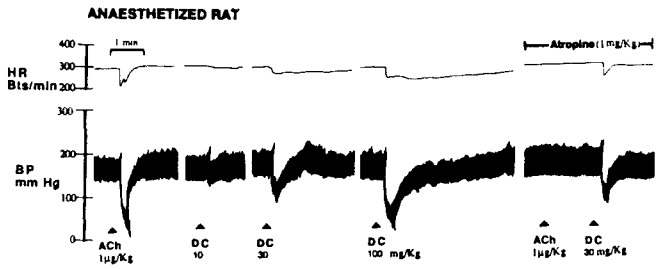

Fig. 1. A representative tracing (n=4) showing comparison of *Daucus carota* (*DC*) and acetylcholine (ACh) for their effects on blood pressure and heart rate in the absence and presence of atropine in normotensive anaesthetized rats. Atropine was administered 5 min before the re-administration of ACh or *DC* responses.

Rabbit Thoracic Aorta: New Zealand white rabbits of either sex weighing 2-3 kg were killed by a blow to the back of the head. The descending thoracic aorta was quickly removed and cut into of 2-3 mm width which were opened by cutting perpendicular to the axis of symmetry of the cylindrical vessel to make strips. Each strip preparation was mounted in a 20 ml tissue bath containing Krebs-Henseleit solution, maintained at 37°C and continuously bubbled with a mixture of 95% oxygen and 5% carbon dioxide. A resting tension of 2 g was applied to each tissue and an equilibrium period of one hour was allowed before changes in isometric tension of the strips were measured as described for atria.

RESULTS

In vivo Studies

In anesthetized rats, ethanolic extract of *Daucus carota* (*DC*) caused a fall in systolic, diastolic and mean arterial blood pressure in a dose-dependent manner. Figure 1 shows tracing from a typical experiment and the combined effect of different experiments have been presented in Table I. At the doses of 10, 30 and 100 mg/kg, *DC* produced decreases of 13, 45 and 59% in mean arterial blood pressure respectively. The hypotensive effect was brief, returning to normal within two minutes. Heart rate was not affected significantly except at the high dose (100 mg/kg) which produced a variable decrease in heart rate (5-15%). Pretreatment of animals with atropine (1 mg/kg) did not abolish the hypotensive response to *DC*, whereas vasodilator and bradycardiac effects of acetylcholine (1 µg/kg) were completely abolished by atropine (Fig. 1). To see whether or not plant extract mediates its hypotensive action through α-adrenoceptors blockade, vasoconstriction was induced with norepinephrine (1 µg/kg). When the arterial blood pressure returned to

Table 1. Effect of ethanolic extract of *Daucus carota* on mean arterial blood pressure (BP) in anesthetized rats (mean± S.E. M.)

Dose (mg/kg)	No. of observations	% Fall in BP
10	4	13.47± 2.28
30	5	44.70± 2.88
100	3	58.50± 5.76

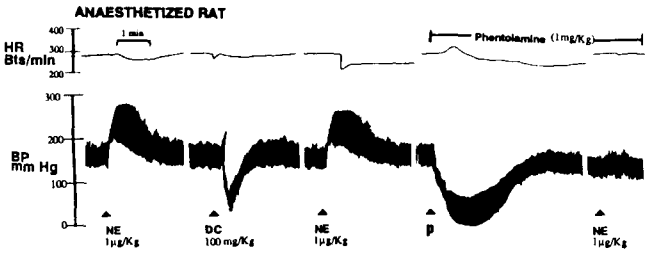

Fig. 2. A representative tracing (n=3) showing comparison of *Daucus carota* (*DC*) and phentolamine (p 1 mg/kg) for their effects on norepinephrine (NE)-induced vasoconstriction in normotensive anesthetized rats.

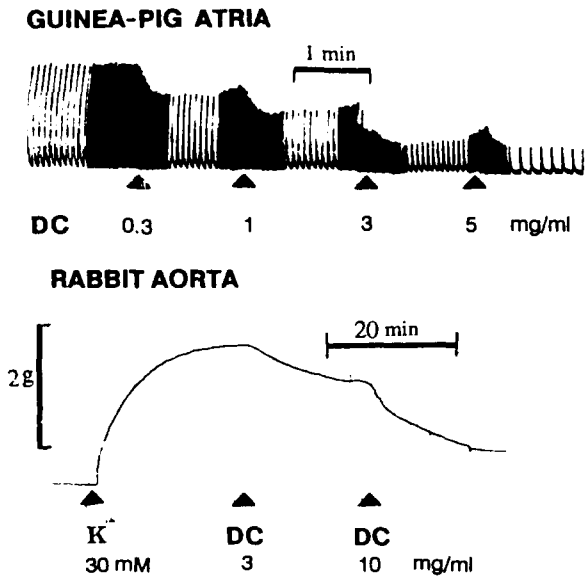

Fig. 3. Representative tracings (n=4) show concentration-dependent inhibitory effects of *Daucus carota* (*DC*) on spontaneously beating guinea-pig paired atria and rabbit aorta pre-contracted with K^+. The time scale for atria represents the low speed and speed of the chart recorder was increased 4 times to record changes in heart rate.

normal values, extract was injected at a dose of 10 mg/kg, the dose previously shown to produce clear hypotensive effect (Fig. 2).

As soon as blood pressure values became stabilized, the same dose of norepinephrine was given again. Figure 2, illustrates that the vasconstrictor effect of norepinephrine before and after the administration of extract was similar, whereas phentolamine (1 mg/kg) abolished the vasconstriction response of norepinephrine.

Effect on Guinea-pig Atria *in vitro*

Ethanolic extract of *Daucus carota* at the concentration range of 0.3-5 mg/ml, caused a progressive decrease in force and rate of atrial contractions in a concentration-dependent manner (Fig. 3a). The inhibitory effect of plant extract was reversible as the tissue regained its original spontaneous contractions 5-10 min after wash out. Pretreatment of tissue with atropine (0.1 µM) did not abolish the inhibitory responses of *DC* both on force and rate of atrial contraction (data not shown).

Effect on Rabbit Aorta *in vitro*

K^+ (30 mM), produced sustained contractions, which allowed the study of the inhibitory effects of the test drugs. When the plateau level was achieved (within 10-20 min), plant extract was then added into the tissue bath in a cumulative fashion (Van Rossum, 1963). Plant extract at the concentration of 3-10 mg/ml, produced a concentration-dependent inhibition of rabbit aorta pre-contracted with K^+ (Fig. 3b). Relaxant effect of plant extract was reversible as the contractile effect of K^+ was restored after washout and repeated administration of spasmogen.

DISCUSSION

The ethanolic extract of *Daucus carota* produced a dose-dependent hypotensive and slight bradycardiac effects in anaesthetized rats. Acetylcholine also produced similar responses which were blocked by atropine, a competitive blocker of acetylcholine at muscarinic receptors (Arunlakshana and Schild, 1959; Gilani and Cobbin 1986, 1987). However, pretreatment of animals with atropine failed to abolish the cardiovascular responses of plant extract in the *in vivo* studies and also bradycardia in isolated atria, suggesting that these effects were not mediated through a mechanism similar to that of acetylcholine. Norepinephrine is a potent vasoconstrictor, mediating this effect through activation of peripheral α-adrenoceptors and produces reflex bradycardia (Hoffman and Lefkowitz, 1990a). Normal tone of the blood vessels is maintained through activation of α-adrenoceptors by the relased norepinephrine and hence α-adrenoceptor blocking drugs produce hypotension (Hoffman and Lefkowitz, 1990b). Phentolamine, an α-adrenoceptor blocking drug (Brown *et al.*, 1980) produced hypotension and abolished norepinephrine-induced vasoconstriction, whereas pretreatment of animals with plant extract did not modify

norepinephrine response on blood pressure. This suggests that unlike phentolamine, plant extract does not mediate its hypotensive effect through α-adrenoceptor blockade. The plant may have direct relaxant action on mycoardium and blood vessels.

This speculation was confirmed when plant extract was tested on isolated smooth muscle preparations. The plant extract inhibited K^+-induced contraction in rabbit aorta, showing its smooth muscle relaxant activity. The contractions of smooth muscle preparations including rabbit aorta is dependent upon an increase in the cytoplasmic free Ca^{2+} which activates the contractile elements (Karaki and Wiess, 1988). The increase in the intracellular Ca^{2+} is due to either influx via voltage-dependent Ca^{2+} channels (VDCs) or release from intracellular stores in sarcoplasmic reticulum (Godfraind *et al.*, 1984). The contraction of rabbit aorta induced by high K^+ is dependent upon ingress of Ca^{2+} into the cells through VDCs (Bolton, 1979). The inhibition of high K^+-induced contraction of rabbit aorta by the plant extract may be visualized as an outcome of restricted Ca^{2+} entry via VDCs.

Thus the results of this study indicate that the direct depressant effects of ethanolic extract of *Daucus carota* may be through blockade of VDCs and that this action is probably responsible for its hypotensive and bradycardiac effects observed in anesthetized rats.

ACKNOWLEDGEMENTS

This study was supported in part by the National Scientific Research Development Board. We would also like to thank Dr. Shaheen Faizi (HEJ Research Institute of Chemistry)for the supplies of plant material.

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