α-Adrenergic Receptor Mediated Hypertensive and Vasoconstrictor Effects of Dietary Radish Leaves Extract

Muhammad Nabeel Ghayur\textsuperscript{a,b} and Anwarul Hassan Gilani\textsuperscript{a,*}

\textsuperscript{a}Department of Biological and Biomedical Sciences, Aga Khan University Medical College, Karachi-74800, Pakistan and \textsuperscript{b}Department of Medicine, McMaster University, St. Joseph’s Hospital, Hamilton L8N 4A6, Ontario, Canada

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Radish is a universally known food plant. Leaves of radish are widely used for their culinary and medicinal properties. In this study we report the hypertensive and vasoconstrictor effects of the aqueous crude extract of radish leaves (Rl.Cr) mediated possibly via activation of α-adrenergic receptors. Rl.Cr, that tested positive for the presence of saponins and alkaloids, exhibited a dose-dependent (10–300 mg/kg) hypertensive effect in the blood pressure (BP) of rats under anaesthesia. This effect was blocked in the presence of phentolamine (2 mg/kg), similar to norepinephrine (NE) a standard adrenergic agonist. Further investigations were conducted on the isolated tissue preparations. In rabbit aorta, Rl.Cr showed a dose-dependent (0.3–5 mg/ml) vasoconstrictor effect that was sensitive to phentolamine pretreatment. Phentolamine pretreatment also shifted the curves of Rl.Cr in a parallel manner to the right in aorta, similar to NE, thus possibly confirming the adrenergic receptor mediated effect. However, Rl.Cr was devoid of any activity in isolated guinea pig atria up to the dose of 10 mg/ml indicating that the extract has a specific α-adrenergic effect independent of β-adrenergic receptor involvement. Thus the study shows hypertensive and vasoconstrictor activities of radish leaves extract possibly mediated via interaction at α-adrenergic receptors.

Key words —— radish leaves, α-adrenergic, hypertensive, vasoconstrictor

INTRODUCTION

\textit{Raphanus sativus} Linn. (Cruciferae), commonly known as ‘radish’, is a popular vegetable plant known and used by people all over the world for its culinary properties. Radish, along with peas and turnip, is considered to be the oldest cultivated crop in the world. It is usually eaten as salad, raw, in its original form, cooked, as a snack or as an appetizer. Apart from its use in the kitchen, different parts of this plant such as the leaves, seeds, and roots are prescribed by traditional healers in South Asia in a variety of disorders of gastrointestinal, cardiovascular, biliary, hepatic, urinary and respiratory origin.\textsuperscript{1, 2} Research has shown that the plant contains antiurolithic,\textsuperscript{3} anti-inflammatory and antibleeding,\textsuperscript{4} influenza protective,\textsuperscript{5} antimicrobial\textsuperscript{6} and antioxidant properties.\textsuperscript{7}

Recently we have shown that the aqueous extract of radish leaves stimulate intestinal smooth muscles of experimental animals via multiple pathways,\textsuperscript{8, 9} both \textit{in vivo} and \textit{in vitro}, one reason why these radish leaves are so popularly used to relieve constipation and delayed motility. In this study, we report the hypertensive and vasoconstrictor activities of the aqueous extract of radish leaves possibly mediated via interaction with α-adrenergic receptors in experimental animals.

MATERIALS AND METHODS

Drugs and Standards —— The following reference chemicals were obtained from the sources specified: acetylcholine chloride (ACh), isoprenaline bitartrate, norepinephrine bitartrate (NE) and phentolamine hydrochloride (Sigma Chemical Company, St. Louis, MO, U.S.A.). The following chemicals were used to make the physiological salt solutions: potassium chloride (Sigma Chemical Company), calcium chloride, glucose, magnesium sulphate, potassium dihydrogen phosphate, sodium bicarbonate and sodium chloride (E. Merck, Darmstadt, Germany). All chemicals used were of the
highest purity grade. Stock solutions of all the chemicals were made in saline and the dilutions were made fresh on the day of the experiment.

**Animals** —— Experiments performed complied with the rulings of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council and were approved by the Aga Khan University’s Ethics Committee for Research on Animals. Sprague-Dawley rats (170–200 g), local rabbits (1 kg) and guinea pigs (500–600 g) of either sex were used in the study and were housed in the animal house of Aga Khan University under a controlled environment (23–25°C). Animals were given tap water *ad libitum* and a standard diet consisting of (g/kg): flour 380, fibre 380, molasses 12, NaCl 5.8, nutrivet L 2.5, potassium metabisulphate 1.2, vegetable oil 38, fish meal 170 and powdered milk 150.

**Plant Material and Extract Preparation** —— Fresh radish leaves (1590 g) were purchased from the main vegetable market in Karachi. A sample of the plant material was deposited at the Herbarium of Department of Biological and Biomedical Sciences, Aga Khan University, Karachi with the voucher # RS-LF-06-02-47. Radish leaves were washed and then soaked in 7 litres of distilled water for a total of 3 days. The plant material was filtered through a piece of porous cloth and then through Whatman qualitative grade-1 filter paper. The filtrate was later shown to yield a thick, green extract (Rl.Cr) weighing 61.06 g with a yield of 3.84% (w/w).

**Preliminary Phytochemical Analysis** —— Rl.Cr was screened qualitatively using various organic solvents and reagents for the presence of different classes of phyto-constituents such as saponins, flavonoids, tannins, phenols, coumarins, sterols, terpenes, alkaloids and anthraquinones. Briefly, saponins were detected on observance of any froth formation following rigorous shaking of the extract dissolved in distilled water. Testing for flavonoids required mixing the extract with AlCl₃ and the appearance of yellow colouration indicated a positive test. Presence of phenols and tannins was determined after the appearance of any green or dark green colour after dissolution of extract in aqueous FeCl₃. For detecting coumarins, a piece of filter paper was moistened in NaOH and then kept over a test tube with boiling plant extract solution. If the filter paper later showed any yellow fluorescence under UV light, that indicated a positive test for coumarins. Detection for sterols and terpenes in the extract involved treating the extract with petroleum ether and then extracting with CHCl₃. The subsequently acquired CHCl₃ layer was treated with acetic anhydride and concentrated HCl. The appearance of pink to purple and green to pink colours was indicative of the presence of terpenes or sterols, respectively. Alkaloids were screened by mixing the extract with Dragendorff’s reagent. Lastly, for detecting anthraquinones, the extract was dissolved in 1% HCl, then in benzene and later, if the extract showed a pink, violet or red colour with NH₄OH, that indicated a positive test of the presence of anthraquinones.

**Blood Pressure (BP) in Anaesthetized Rats** —— The experiment was performed as previously described. Rats were anaesthetized with an intraperitoneal injection of sodium thiopental (Pentothal, 70–90 mg/kg body weight). When light anaesthesia was achieved, the right carotid artery was cannulated by polyethylene tubing PE-50, which was connected to a pressure transducer (P23 XL) coupled with a Grass model 7 polygraph. This connection was used for BP recording. The left jugular vein was cannulated with similar tubing to facilitate the intravenous injection of the drugs and plant material. The exposed surface of the cannulation was covered with cotton wool moistened in warm saline. After a 20 min period of equilibrium, the rats were injected intravenously with 0.1 ml saline (NaCl 0.9%) or with the same volume of radish extract. Arterial BP was allowed to return to the resting level between injections. Standard drugs and the radish leaf extract (all prepared in saline) were then administered by i.v. injections and flushed in with 0.1 ml saline. Control responses of standards such as ACh (1 µg/kg) and NE (1 µg/kg) were obtained before testing the extract. Changes in BP were recognized as the difference between the steady state values before and the lowest readings after injection. Mean arterial blood pressure (MABP) was calculated as the diastolic BP plus one-third pulse width.

**Isolated Rabbit Aorta** —— Isolated tissue experiments were carried out as previously described. Rabbits were sacrificed by cervical dislocation. 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 at 37°C and aerated with 5% carbon dioxide in oxygen (carbogen). The composition of Kreb’s
solution was (mM): NaCl 118.2, NaHCO₃ 25.0, CaCl₂ 2.5, KCl 4.7, KH₂PO₄ 1.3, MgSO₄ 1.2 and glucose 11.7 (pH 7.4). A resting tension of 2 g was applied to each tissue and an equilibrium period of 1 hr was allowed before studying the effect of radish leaf extract. The changes in isometric tensions of the rings were measured via a force-displacement transducer (FT-03) using a Grass model 7 polygraph. To learn if the extract had any effect, it was determined on the resting baseline of the tissue.

**Isolated Guinea pig Atria** —— Right and left atria from guinea pigs were dissected, removed and mounted separately in 20 ml tissue baths containing Kreb’s solution at 32°C then aerated with carbogen. The tissues were allowed to beat spontaneously under the resting tension of 1 g. An equilibrium period of 30 min was given before the application of any drug. Control responses of isoprenaline (0.1 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.

**Statistical Analysis** —— All the data expressed are mean ± standard error of mean (SEM, n = number of experiment) and the median effective concentrations (EC₅₀) with 95% confidence intervals (CI). Statistical parameter applied is one way analysis of variance (ANOVA) followed by Tukey’s multiple comparison test and two-way ANOVA with P < 0.05 noted as significantly different (GraphPAD program, GraphPAD, San Diego, CA, U.S.A.).

**RESULTS AND DISCUSSION**

When Rl.Cr was tested *in vivo* on the BP of rats under anaesthesia, it exhibited a dose-dependent (30–300 mg/kg) rise in MABP of nor-motensive anaesthetized rats with an EC₅₀ value of 218.4 mg/kg (64.9–734.5, 95% CI, n = 4). NE (1 µg/kg), a standard adrenergic agonist and hypertensive agent, also showed a hypertensive effect on BP. The effect of Rl.Cr was brief and returned to control values within a minute. Figure 1 shows the tracing of a typical experiment while Fig. 2 shows a bar diagram representing the combined results of different experiments. Pretreatment of animals with phentolamine (2 mg/kg), a standard α-adrenergic antagonist, completely blocked the hypertensive effect on BP. The effect of Rl.Cr was brief and returned to control values within a minute. Figure 1 shows the tracing of a typical experiment while Fig. 2 shows a bar diagram representing the combined results of different experiments. Pretreatment of animals with phentolamine (2 mg/kg), a standard α-adrenergic antagonist, completely blocked the hypertensive effect of the extract similar to that of NE (Fig. 1).

The α-adrenergic effect of the extract was further evaluated in an isolated rabbit aorta preparation where Rl.Cr produced a dose-dependent (0.1–5 mg/ml) vasoconstrictor effect (Fig. 3) when tested on the resting baseline of the tissue, with an EC₅₀ value of 1.3 mg/ml (0.6–1.9, n = 4). The extract achieved its highest contractile effect of 82.3 ± 4.1% (n = 4) of the NE maximum (Fig. 4A). The effect of lower doses of Rl.Cr (0.1–1 mg/ml) was abolished while effect of higher doses (3–5 mg/ml) was substantially blocked in the presence of phentolamine (3 µM; Fig. 4A). Sub-maximal responses (1 µM) of NE were also blocked in the presence of the antagonist (Fig. 3). However, a critical look at
Fig. 2. Bar Diagram Showing the Hypertensive Effect of Radish Leaves Crude Extract (RL.Cr) in Anaesthetized Rats (Values Shown are Mean ± SEM, n = 4; One-way ANOVA Followed by Tukey’s Multiple Comparison Test).

Fig. 3. Typical Tracing Showing the Effect of Radish Leaves Crude Extract (RL.Cr) in Comparison to Isoprenaline (Iso) in Isolated Guinea Pig Atria and in Comparison to Norepinephrine (NE) in the Absence and Presence of Phentolamine (3 µM) in Isolated Rabbit Aorta.

Fig. 4. Dose-response Curves Showing the Effect of (A) Radish Leaves Crude Extract (RL.Cr) and (B) Norepinephrine (NE) in the Absence and Presence of Phentolamine (Phent, 3 µM) in Isolated Rabbit Aorta (Values Shown are Mean ± SEM, n = 4; Both the Curves and Individual Doses in [A] and [B] are Significantly Different from Each Other, P < 0.001, Two-way ANOVA).

this in vitro effect of RL.Cr revealed that there was some dissimilarity with the effect shown by NE. As evident from the tracing (Fig. 3), the effect shown by the extract was slow and not mediated in a prompt manner as exhibited by NE. Furthermore, phentolamine pretreatment partially blocked the vasoconstrictor response of the plant extract as opposed to complete blockade of NE response (Fig. 3). These same observations for the onset of action and degree of blockade by the antagonist can again be made in the dose-response curves for the vasoconstrictor effect of RL.Cr and NE in the absence and presence of phentolamine in the aortic preparations (Fig. 4). Both the effect of RL.Cr (Fig. 4A) and NE (Fig. 4B) were displaced to the right in a parallel manner (P < 0.001), without suppression of the maximum effect in the presence of phentolamine, indicating competitive interaction at the α-adrenergic receptors.16)

Norepinephrine is also known to produce cardiac stimulatory responses through the activation of β1-adrenergic receptors.13) To find out whether or not RL.Cr behaved like NE on the cardiac tissues, it was tested on the isolated guinea pig atrial preparations. RL.Cr was found devoid of any activity (Fig. 3) on the force or rate of atrial contractions when tested up to the dose of 10 mg/ml (n = 4), indicating that it has no effect on β1-adrenergic receptors.
Phytochemical analysis performed showed the presence of saponins and alkaloids in RL.Cr indicating that they might be responsible for the observed activity from the crude extract, as the saponins and alkaloids are reported to have vasoconstrictor effects in vascular tissues.\(^\text{17, 18}\)

The results show hypertensive and vasoconstrictor effects of the radish leaves crude extract on the blood pressure of rats and vascular tissues from rabbit, possibly mediated through activation of \(\alpha\)-adrenergic receptors. The effect on blood pressure observed in rodents appears to be mediated at relatively large doses, however, in view of the fact that small animals like rats and mice are known to have a more rapid metabolism (around 10 times faster) than humans,\(^\text{19}\) the dose in humans for the hypertensive effect might have been close to 30 mg/kg. For a person of average build (under 70 kg), the amount of radish that might elicit a hypertensive effect could be roughly around 2 g. This mild effect observed in rodents may not be of great concern for healthy individuals, however, it points out an important observation which may have some impact on borderline hypertensive subjects using radish on a regular basis or those who are on antihypertensive medication (around 10 times faster) than humans,\(^\text{19}\)

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REFERENCES