Available online at www.sciencedirect.com

SCIENCE DIRECT

Vascular Pharmacology 43 (2005) 234 – 241

Vascular Pharmacology

www.elsevier.com/locate/vph

Cardiovascular effects of ginger aqueous extract and its phenolic constituents are mediated through multiple pathways

Muhammad Nabeel Ghayur [a,b], Anwarul Hassan Gilani [a,*], Maria B. Afridi [a], Peter J. Houghton [b]

[a] *Department of Biological and Biomedical Sciences, The Aga Khan University Medical College, Karachi 74800, Pakistan*
[b] *Pharmacognosy Research Lab, Department of Pharmacy, Franklin-Wilkins Building, King's College London, London SE1 9NN, UK*

Received 17 January 2005; received in revised form 1 April 2005; accepted 28 July 2005

Abstract

Ginger is a world known food plant which is equally reputed for its medicinal properties. We report here the hypotensive, endothelium-dependent and independent vasodilator and cardio-suppressant and stimulant effects of its aqueous extract (Zo·Cr). Zo·Cr, which tested positive for saponins, flavonoids, amines, alkaloids and terpenoids, induced a dose-dependent (3.0–10.0 mg/kg) fall in the arterial blood pressure (BP) of anaesthetized rats which was partially blocked by atropine (1 mg/kg). In isolated endothelium-intact rat aorta, Zo·Cr (0.01–5.0 mg/ml) relaxed the phenylephrine (1 μM)-induced contractions, effect partially blocked by atropine (1 μM). Zo·Cr inhibited the K^+ (80 mM)-induced contractions and also shifted the Ca^{++} dose-response curves to the right, similar to verapamil, indicating Ca^{++} antagonist activity. An atropine-resistant and L-NAME-sensitive vasodilator activity was also noted from ginger phenolic constituents 6-, 8- and 10-gingerol, while 6-shogaol showed a mild vasodilator effect. In guinea-pig atria, Zo·Cr (0.1–5.0 mg/ml) inhibited the force and rate of atrial contractions. Pretreatment with atropine blocked the inhibitory effect and a stimulatory effect was unmasked which was resistant to propranolol and verapamil but sensitive to ryanodine, blocker of Ca^{++} release from intracellular stores. Later at doses $\geq$1.0 mg/ml, the extract completely suppressed the atrial tissue, effect resistant to glibenclamide, pyrilamine, aminophylline and L-NAME. These data indicate that the aqueous ginger extract lowers BP through a dual inhibitory effect mediated via stimulation of muscarinic receptors and blockade of Ca^{++} channels and this study provides sound mechanistic basis for the use of ginger in hypertension and palpitations.

Keywords: Ginger; Hypotensive; Cardioactive; Cholinergic; Calcium antagonist; Gingerol

1. Introduction

Zingiber officinale Roscoe (Zingiberaceae) is a large biennial herb that grows abundantly in South Asia. The rhizome of the plant, commonly known as ginger, is known and used universally for its medicinal and culinary properties. Ginger is well known all over the world especially for its use in disorders of the gastrointestinal tract such as constipation, dyspepsia, diarrhoea, nausea and vomiting (Tyler, 1993). Ginger is also recommended by the traditional healers in South Asia for use in cardiopathy, high blood pressure, palpitations and to improve the circulation for its use as a vasodilator (Kapoor, 1990; Duke, 2002).

Phytochemical reports have shown that the main constituents of ginger are the gingerols, shogaols, zingerone and paradol (Langner et al., 1998). 6-gingerol and 6-shogaol are the major gingerol and shogaol present in the rhizome (Connell and McLachlan, 1972). The main aroma-defining component is zingiberol while others as gingediol, mono-acyldigalactosyl-glycerol, diarylheptanoids and phytosterols have also been identified (Varma et al., 1962).

Ginger has been extensively studied for its biological activities (Langner et al., 1998). Recently, we reported that the 'methanolic extract' of fresh ginger exhibits hypotensive, endothelium-independent vasodilator and cardio-suppressant properties through its specific inhibitory action at the voltage-dependent calcium channels (Ghayur and Gilani,

* Corresponding author. Tel.: +92 21 4859 4571; fax: +92 21 493 4294/493 2095.
E-mail address: anwar.gilani@aku.edu (A.H. Gilani).

1537-1891/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.vph.2005.07.003

2005). In this report, we investigate the effects of the 'aqueous extract' of fresh ginger on the cardiovascular parameters and show that it lowers blood pressure (BP) through cholinergic and calcium channel blocking (CCB) properties and possesses a combination of cardio-suppressant and cardio-stimulant actions in experimental animals. Apart from the crude extract, some of the known pungent principles of ginger namely: 6-gingerol, 8-ginegrol, 10-gingerol and 6-shogaol (Connell and McLachlan, 1972) were also tested on the vascular tissues (due to the limited quantity, they were only screened in aorta) and were found to exhibit a vasodilator effect through a combination of nitric oxide (NO) releasing and calcium antagonist mechanisms.

2. Materials and methods

2.1. Drugs and standards

The following reference chemicals namely: acetylcholine chloride (ACh), atropine sulphate, aminophylline hydrate, glibenclamide, isoprenaline bitartrate, N_{ω}-nitro-L-arginine methyl ester hydrochloride (L-NAME), norepinephrine bitartrate (NE), phenylephrine hydrochloride (PE), propranolol hydrochloride, pyrilamine maleate, ryanodine and verapamil hydrochloride were obtained from the Sigma Chemical Company, St. Louis, MO, USA. Pure compounds: 6-gingerol, 8-gingerol, 10-ginegrol and 6-shogaol were obtained from ChromaDex Inc. Santa Ana, CA, USA. Chemicals used for making physiological salt solutions were: potassium chloride (Sigma Chemical Company, St. Louis, MO, U.S.A.), calcium chloride, glucose, magnesium chloride, magnesium sulphate, potassium dihydrogen phosphate, sodium bicarbonate, sodium chloride and sodium dihydrogen phosphate (E.Merck, Darmstadt, Germany) and ethylenediaminetetra-acetic acid (EDTA) from BDH Laboratory Supplies, Poole, England. Stock solutions of all the chemicals were prepared in distilled water and the dilutions were made fresh on the day of the experiment.

2.2. Animals

Experiments performed complied with the rulings of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (NRC, 1996). Sprague–Dawley rats (180–200 g) and guinea-pigs (500–600 g) of either sex (aorta was isolated only from male rats) used in the study were housed in the animal house of The Aga Khan University under a controlled environment (23–25 °C). Animals were given tap water ad libitum and a standard diet.

2.3. Plant material and extraction

A total of 1 kg of fresh ginger rhizome was purchased from the main vegetable market in Karachi in the month of June. A sample of the rhizome was deposited at the Herbarium of Department of Biological and Biomedical Sciences, The Aga Khan University, Karachi with the voucher # ZO-RH-06-02-46. The plant material was washed for any contaminants and then the rhizome was crushed in a mortar and pestle to expose the inner part. The sample was then soaked in 2 l of distilled water at 20 °C and kept for a total of 3 days. It was then filtered through a porous cloth, the filtrate collected and this procedure was repeated twice. In the end, all of the filtrate was combined, filtered through Whatman qualitative Grade-1 filter paper and then concentrated in a rotary evaporator to obtain a thick, brown extract (Zo·Cr) weighing 22 g (yield of 2.2%).

2.4. Phytochemical screening

The fresh ginger aqueous crude extract (Zo·Cr) was screened for the presence of different classes of compounds by thin layer chromatography using silica gel G (Merck) plates of 0.25mm thickness (Wagner et al., 1984). The extract was dissolved in chloroform: methanol (2:1 v/v) while the development of plates was carried out with chloroform: methanol (3:17 v/v). After development, the plates were sprayed with the following solvents and reagents for detection of the respective classes of compounds: water (lipophilic compounds); sulphuric acid and heating at 105° for 5 min (organic compounds); 0.5% anisaldehyde in sulphuric acid, glacial acetic acid and methanol 5:10:85 v/v (terpenoids); 10% antimony trichloride in chloroform (flavonoids/ terpenoids); 1% diphenylboric acid 2-aminoethyl ester in methanol followed by 5% polyethylene glycol 4000 in 96% ethanol (flavonoids); 0.5% ninhydrin in acetone (amino acids/peptides and secondary amines); 5% ethanolic sodium hydroxide (anthraquinones); 5% aqueous ferric chloride (tannins/phenols); 20% aqueous sodium carbonate followed by Folin-Ciocalteu reagent (phenols); 0.5% aqueous fast blue B salt followed by 0.1 M aqueous sodium hydroxide (phenols); Dragendorrf's reagent (alkaloids) and dilute sodium hydroxide (coumarins). Reagents were prepared according to Stahl (1969). Detection was carried out visually in visible light and under UV light (λ=365 nm). Saponins were detected by observing froth formation by the extract in a test tube after regular shaking while the coloured, double-bonded and fluorescent compounds were detected on the silica gel plates under day light, UV light 254 nm and UV light 365 nm respectively.

2.5. BP in anaesthetized rats

These experiments were performed on adult rats of either sex as described previously (Gilani et al., 1994). The animals 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 and 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 to facilitate the intravenous injection of the drugs and plant material. The temperature of the animals was maintained at 37 °C. After a 20 min period of equilibrium, the rats were injected intravenously with 0.1 ml saline or with the same volume containing the test substance. Standard drugs and the ginger extract (all prepared in saline) were then administered by i.v. injections and flushed in with 0.1 ml saline. Control responses of ACh (1 μg/kg) and NE (1 μg/kg) were obtained. 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.

2.6. Endothelium-intact rat aorta

Thoracic aorta, isolated from male rats, was set up by following the procedure of Furchgott and Zawadski (1980) with slight modifications. Individual aortic rings were mounted in 5 ml tissue baths, with Kreb's solution at 37 °C, aerated with carbogen and allowed to incubate for 30 min under the resting tension of 1 g. The composition of Kreb's solution in mM was: NaCl 118.2, $NaHCO_3$ 25.0, $CaCl_2$ 2.5, KCl 4.7, KH_2PO_4 1.3, $MgSO_4$ 1.2 and glucose 11.7 (pH 7.4). Changes in tension were recorded via World Precision Instrument's (WPI) Isometric Force transducers (Fort 100). Effect of the extract and its pure compounds was firstly observed on the resting baseline to detect any vasoconstrictor effect and later on PE (1 μM)-induced contractions, only after confirmation of presence of endothelium with ACh (0.1–0.3 μM). Tissues were preincubated with atropine (1 μM) and L-NAME (0.1 mM) for studying a cholinergic or NO-releasing endothelium-dependent mechanism of vasodilation. The calcium channel blocking (CCB) effect was assessed by testing on high K^+ (80 mM)-induced contraction (Bolton, 1979). To confirm the CCB effect, the tissue was allowed to stabilize in normal Kreb's solution, which was then replaced with Ca^{++}-free Kreb's solution containing EDTA (0.1 mM) for 30 min in order to remove calcium from the tissues. This solution was further replaced with K^+-rich and Ca^{++}-free Kreb's solution, having the following composition: KCl 50, NaCl 91.04, $MgSO_4$ 1.05, $NaHCO_3$ 11.90, glucose 5.55 and EDTA 0.1 mM. Following an incubation period of 30 min, control dose-response curves of Ca^{++} were obtained and then redetermined after pretreating the tissue for 60 min with the plant extract or verapamil (positive control).

2.7. Guinea-pig atria

Right atria isolated from guinea-pigs were mounted separately in 20 ml tissue baths containing Kreb's solution (concentration of components given above) at 32 °C (unsteady recording at >32 °C) and aerated with carbogen gas (Gilani et al., 1994). The tissues were allowed to beat spontaneously under a resting tension of 1 g. An equilibrium period of 30 min was given before the application of any drug. Control responses of ACh (0.1–0.3 μM) and isoprenaline (0.3 μM) were obtained as tension changes in the tissue were recorded via a Grass force-displacement transducer (model FT-03) using Grass Polygraph.

2.8. Data analysis and statistics

All the data expressed are as mean ± standard error of mean (SEM, n = number of experiments) and the median effective concentrations (EC_{50} values) with 95% confidence intervals (CI). The statistical parameter applied is the Student's t-test (paired or unpaired) with $P<0.05$ noted as significantly different (GraphPAD program, GraphPAD, San Diego, CA, USA). Concentration-response curves were analysed by non-linear regression (GraphPAD program).

3. Results

3.1. Phytochemical screening

Zo·Cr was found to contain double-bonded, fluorescent, lipophilic and organic compounds while saponins, terpe-

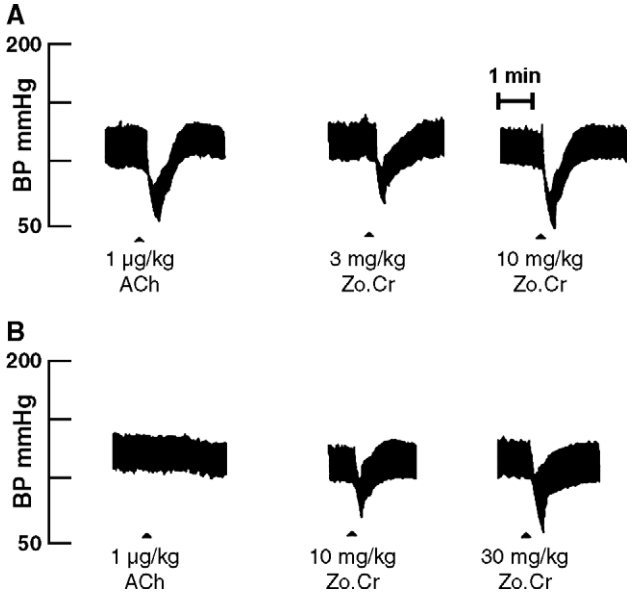

Fig. 1. Typical tracing showing the hypotensive effect of increasing doses of ginger aqueous crude extract (Zo·Cr) in comparison to acetylcholine (ACh) in: (A) absence and (B) presence of atropine (1 mg/kg) on mean arterial blood pressure (BP) of anaesthetized rats. The extract dose of 3 mg/kg was completely blocked in the presence of atropine thus is not shown in (B).

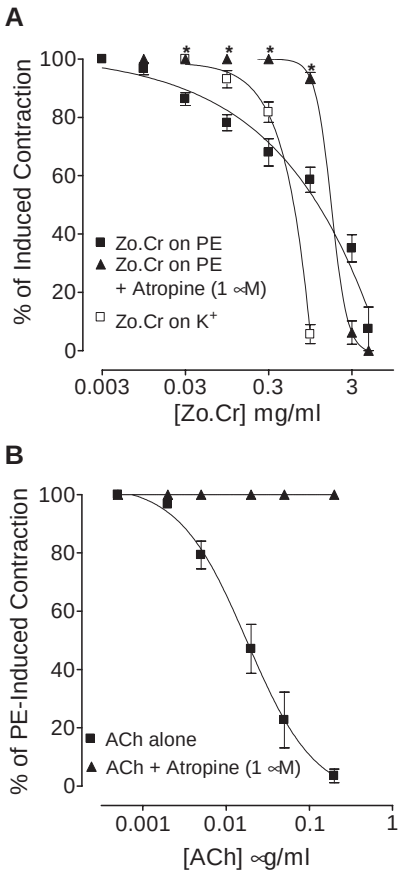

Fig. 2. Dose-response curves showing the effect of (A) ginger aqueous crude extract (Zo·Cr) on phenylephrine (PE, 1 μM)-induced contractions in the absence and presence of atropine (black symbols) and on K^+ (80 mM)-induced contractions (hollow symbols) in isolated endothelium-intact rat aorta. Lower panel (B) shows the vasodilator effect of acetylcholine (ACh) on PE (1 μM)-induced contractions in the absence and presence of atropine in rat aorta. Values shown are mean±SEM, $n=4$; *$P<0.001$ vs. 'Zo·Cr on PE' curve values, Student's t-test.

noids, flavonoids, amino acids/peptides, secondary amines and alkaloids were also present in the crude extract. The extract did not test positive for the other classes of compounds.

3.2. Effect on BP in anaesthetized rats

Zo·Cr induced a dose-dependent (3–10 mg/kg) fall in the BP of rats under anaesthesia (Fig. 1, $n=3$). The doses of 3 and 10 mg/kg induced a respective fall of 33.3±0.8% and 46.9±0.9% (mean±SEM) in the BP. When this hypotensive effect of Zo·Cr was challenged with atropine (1 mg/kg), partial blockade was observed (Fig. 1), with almost complete blockade of the effect observed at 3 mg/kg dose of the extract (7.4±1.7%, $P<0.001$) while the effect at the next higher dose (10 mg/kg) was partially blocked (31.4±2.1%, $P<0.01$). The dose of 30 mg/kg produced an effect (38.1±1.3%), in the presence of atropine, that was comparable to that of 10 mg/kg in the absence of atropine. ACh (1 μg/ml) also showed a hypotensive effect which was completely blocked in the presence of atropine as expected (Fig. 1).

3.3. Effect on endothelium-intact rat aorta

When tested on the resting baseline of rat aorta, Zo·Cr was found devoid of any vasoconstrictor activity below 10 mg/ml. Later contraction was induced with PE (1 μM) to allow testing for an inhibitory effect. The extract in increasing doses (0.01–5.0 mg/ml) depressed this induced contraction (Fig. 2A) with an EC_{50} value of 1.2 mg/ml (0.6–1.8, 95% CI, $n=4$). Atropine (1 μM) blocked the vasodilator effect of the lower doses (0.01–1.0 mg/ml) of Zo·Cr ($P<0.001$), changing the slope of the curve, with an EC_{50} value of 1.7 mg/ml (1.4–1.9, $n=4$). Unlike the extract, vasodilator effect of ACh was completely abolished in the presence of atropine (Fig. 2B). When tested on high K^+ (80 mM)-induced contraction, in the absence of atropine, Zo·Cr dose-dependently (0.1–1.0 mg/ml) relaxed the sustained contraction (Fig. 2A) with an EC_{50} value of 0.5 mg/ml (0.4–0.6, $n=4$). The extract dose-dependently (0.1–0.3 mg/ml) shifted the Ca^{++} dose-response curves to the right (Fig. 3A) with suppression

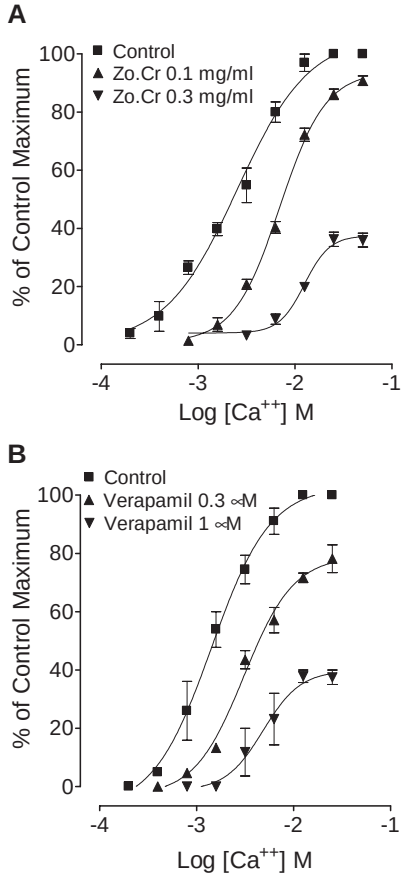

Fig. 3. Dose-response curves showing the effect of increasing doses of (A) ginger aqueous crude extract (Zo·Cr) and (B) verapamil on Ca^{++} dose-response curves constructed in Ca^{++} free medium in isolated rat aorta (values shown are mean±SEM, $n=4$).

of the maximum effect, similar to an effect produced by verapamil (Fig. 3B).

The known pure compounds from ginger were also tested in rat aorta preparations. All the four compounds were found devoid of any stimulant effect on the resting baseline of the vascular preparation when tested up to the doses of 1 mg/ml. All the gingerols (6-gingerol, 8-gingerol and 10-gingerol) when tested on PE (1 μM)-induced contractions, exhibited a dose-dependent (1.0–300 μg/ml) vasodilator effect (Fig. 4) with respective EC_{50} values of 24.9 μg/ml (15.1–41.2, $n=3$), 90.9 μg/ml (56.0–147.7, $n=4$) and 32.5 μg/ml (20.6–51.4, $n=3$). 6-Shogaol, however, was unable to exhibit any significant vasodilator effect on the agonist-induced contractions (Fig. 4D). The vasodilator effect of all the gingerols was resistant to blockade by atropine (1 μM) but was considerably blocked in the presence of L-NAME (0.1 mM). The blockade of the vasodilator effect with L-NAME was maximum with 10-gingerol (Fig. 4C). Of all the gingerols, only 6-gingerol was tested on high K^+ (80 mM)-induced contractions, due to the limited supply of the other compounds, and was found to exhibit an inhibitory effect (Fig. 4A) with an EC_{50} value of 90.4 μg/ml (77.9–104.9, $n=3$).

3.4. Effect on guinea-pig atria

In isolated guinea-pig atria, Zo·Cr exhibited a dose-dependent (0.1 to 5.0 mg/ml) inhibitory effect on the force of spontaneous atrial contractions (Fig. 5A) with an EC_{50} value of 0.84 mg/ml (0.3–2.2, $n=4$). Increasing the spread of the chart paper showed that the extract also inhibited the rate of atrial contractions observed in one experiment (data not shown). Only the inotropic effect was tested in the subsequent experiments as the system did not allow simultaneous recordings of heart rate. Pretreatment of the tissue with atropine (1 μM) blocked the inhibitory effect of Zo·Cr and a cardio-stimulant effect at lower doses was unmasked which was followed by relaxation at the higher doses (≥1.0 mg/ml, Fig. 5A). This stimulatory effect of the extract was insensitive to propranolol (1 μM) and verapamil (1 μM) pretreatment (Fig. 5B). Similarly, it did not potentiate the stimulatory effect of isoprenaline (0.3 μM) (data not shown). The cardio-stimulant effect of Zo·Cr was

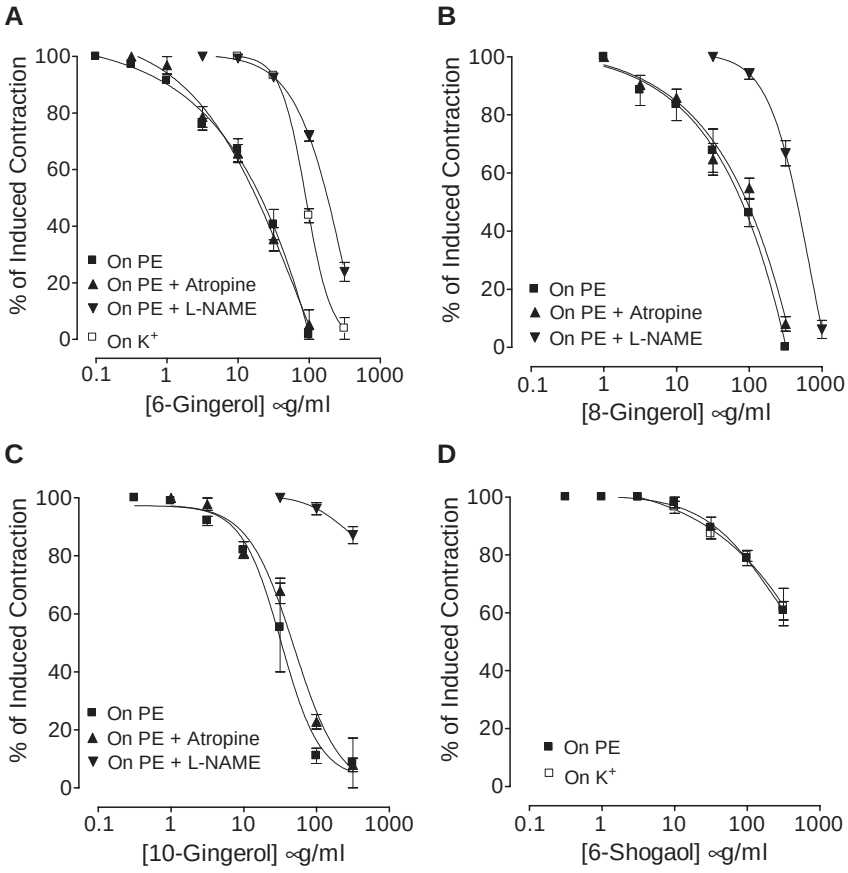

Fig. 4. Dose-response curves showing the vasodilator effect of the ginger active ingredients: (A) 6-gingerol, (B) 8-gingerol, (C) 10-gingeroal and (D) 6-shogaol in the absence and presence of the atropine (1 μM) and N_ω-nitro-L-arginine methyl ester (L-NAME, 1 mM) on phenylephrine (PE 1 μM, dark symbols) and K^+ (80 mM, hollow symbols)-induced contractions in isolated endothelium-intact rat aorta preparations. Due to limited supplies, only 6-gingerol and 6-shogaol could be tested on K^+-induced contractions. Values shown are mean ± SEM, $n=3–4$.

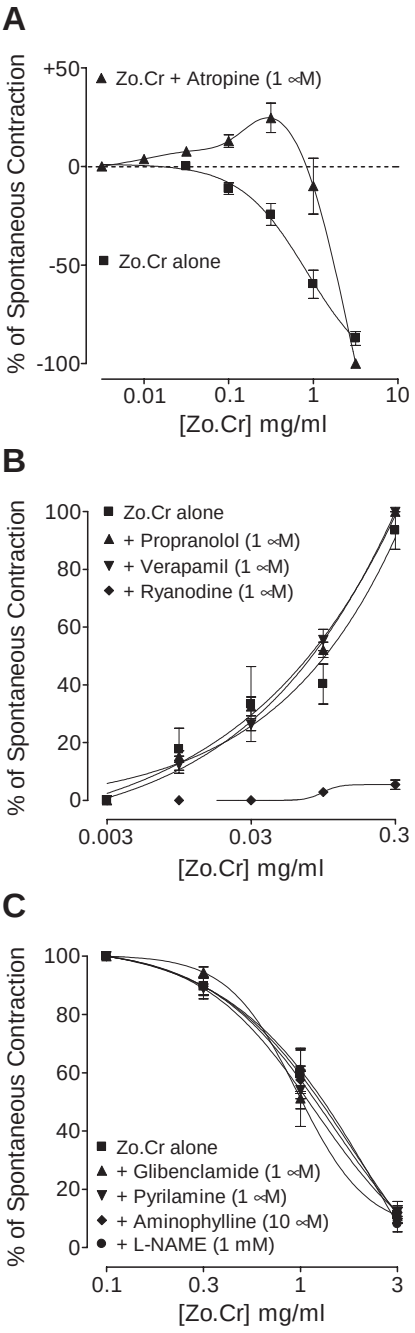

Fig. 5. Dose-response curves showing the effect of ginger aqueous crude extract (Zo·Cr) on guinea-pig atrial force of contraction: (A) in absence and presence of atropine (1 μM), (B) +ve inotropic effect (in atropinized preparations) challenged with propranolol (1 μM), verapamil (1 μM) and ryanodine (1 μM) and (C) −ve inotropic effect (in atropinized preparations) challenged with glibenclamide (1 μM), pyrilamine (1 μM), aminophylline (10 μM) and L-NAME (1 mM). Values shown are mean ± SEM, $n = 4–6$.

blocked in the presence of ryanodine (1 μM, Fig. 5B). The relaxant effect (Fig. 5C), following the cardio-stimulation, was found to be resistant to pretreatment of the tissue with glibenclamide (1 μM), pyrilamine, aminophylline (10 μM) and L-NAME (1 mM).

4. Discussion

The observed fall in BP of normotensive rats under anaesthesia is in line with the traditional use of ginger as an antihypertensive and vasodilator agent (Kapoor, 1990; Diamond, 2001; Duke, 2002). The partial blockade with atropine, a competitive muscarinic antagonist (Arunlakhshana and Schild, 1959; Gilani and Cobbin, 1986), indicated presence of a cholinergic and an additional component in the BP lowering effect of ginger. Cholinergic compounds are known to cause a fall in BP by activation of muscarinic receptors located on the epithelium of blood vessels (Furchgott and Zawadski, 1980).

Ginger has not been reported earlier for its blood pressure lowering effects. In our earlier report (Ghayur and Gilani, 2005), we observed that a 'methanolic' extract of fresh ginger exhibits hypotensive effect in anesthetized rats via blockade of Ca^{++} channels while here in this investigation we report a cholinergic receptor mediated hypotensive activity of 'aqueous' ginger extract. The difference in activity could be a result of the different solvent systems used for extraction as it is known that organic solvents extract non-polar while distilled water would extract polar compounds (Harborne, 1984; Williamson et al., 1996). These results are in line with our previous studies showing that the cholinergic component is usually concentrated in the aqueous while the CCB component is concentrated in the less polar extracts (Gilani et al., 2000a,b, 2005). Interestingly, the potency of the CCB activity was less in this study when compared with the earlier study carried out with the ginger methanolic extract (Ghayur and Gilani, 2005). A difference in activity was also reported between methanolic and aqueous ginger extracts for their vascular effects by Pancho et al. (1989). On the other hand, Weidner and Sigwart (2000) showed that an 'ethanol extract of dried ginger' was devoid of any effect on the BP or heart rate of 'conscious rats'. This could possibly be due to a different solvent used for extraction (ethanol), using conscious instead of anaesthetized animals or using 'dried ginger' which has a chemical profile significantly different from fresh ginger as the process of drying leads to transformation of certain chemicals due to dehydration (Suekawa et al., 1984, 1986a; Pancho et al., 1989). The results obtained on dried ginger extract by Weidner and Sigwart (2000) appear to be less meaningful particularly when in the same study these authors failed to reproduce the hypoglycaemic effect of ginger earlier obtained by Mascolo et al. (1989).

Apart from ginger itself, some of ginger pure compounds, 6-gingerol and 6-shogaol have been studied for their effects on BP in laboratory animals (Suekawa et al., 1984) where both were found to produce a depressant effect, at lower doses, and a tri-phasic effect (consisting of an initial hypotensive followed by a sharp hypertensive and then a delayed hypotensive effect), at the higher doses, in rats under anaesthesia. Later, reports revealed that the peripheral pressor effect of 6-shogaol in rats is caused by release of a

peptide-like substance from the sympathetic nerve endings (Suekawa et al., 1986a,b).

To further investigate the mechanism of the hypotensive response from the extract, rat aorta was selected which is a prototype tissue used for evaluating different vasodilator mechanisms (Ajay et al., 2003). Zo·Cr dose-dependently relaxed the PE-induced contractions, similar to ACh. When these effects were challenged with atropine, ACh was completely blocked while Zo·Cr was partially blockade indicating presence of an additional vasodilator component. This is the first report of endothelium-dependent vasodilation from ginger. Cholinergic receptor mediated vasodilation is due to release of NO from the endothelium (Furchgott and Zawadski, 1980) and consequent increase of cGMP contents in the vascular smooth muscles in response to activation of guanylyl cyclase (Andreopoulos and Papapetropoulos, 2000). The other vasodilator component mediated by the aqueous ginger extract was due to CCB, as it relaxed the high K^+-induced contractions specifically (Bolton, 1979; Mecca and Love, 1992) as well as shifted the Ca^{++} dose-response curves to the right (Godfraind et al., 1986; Karaki et al., 1997).

Some of the known pungent constituents of ginger namely: 6-gingerol, 8-gingerol, 10-gingerol and 6-shogaol (Connell and McLachlan, 1972) were also tested in the endothelium-intact rat aorta. It was found that the gingerols were more active than 6-shogaol for the vasodilator activity. 6-Shogaol exhibited only a mild vasodilator effect on the PE and high K^+-induced contractions. The observed vasodilator effect of gingerols was insensitive to atropine pretreatment but considerable blockade was observed in the presence of L-NAME, an NO synthase inhibitor (Thorin et al., 1998), as evidenced by the rightward shift in the curves of all the gingerols in the presence of the antagonist. 6-Gingerol was also able to relax the high K^+-induced contractions indicating the co-existence of a Ca^{++} antagonist-type component (Bolton, 1979). 6-Gingerol has been reported for an inhibitory response on the spontaneous contractions and Ca^{++} spikes in isolated portal veins of mice (Kimura et al., 1988) thus supporting the conclusion of a Ca^{++} antagonist action. Unlike the crude extract, no cholinergic involvement was seen in the vasodilator effect of the ginger compounds. It could be possible that the cholinergic-mediated vascular effects of ginger are due to sesquiterpenes such as zingiberene, bisabolene, camphene, and phellandrene (none of which were tested in this study due to their commercial unavailability) also known to be present in ginger (Langner et al., 1998). Alternatively, it is possible that the compound(s) with cholinergic activity is yet to be isolated.

Cholinergic receptors are known to be present in guinea-pig atria (Blinks, 1965) incurring an inhibitory effect (Caulfield, 1993) while the blockade of Ca^{++} channels in the cardiac muscles is also linked to relaxation of the tissue (Karaki et al., 1997). Keeping in mind that the extract possesses ACh-like and CCB activities, a generalized relaxant activity of Zo·Cr in guinea-pig atria was expected. When tested on the guinea-pig atria, Zo·Cr inhibited the spontaneous atrial contractions, an effect which was blocked by atropine suggesting that the cholinergic component is dominant in eliciting the inhibitory effect in heart. This is the first report of a cholinergic mediated cardio-suppressant effect of ginger. The blockade by atropine was followed by a stimulant effect at the similar doses followed by complete relaxation of the tissue at high doses. The cardiotonic effect of the extract was not blocked by propranolol or verapamil ruling out the possibility of β-adrenergic receptors or Ca^{++} channels. Similarly, it did not potentiate the cardio-stimulatory effect of isoprenaline ruling out the involvement of phosphodiesterase inhibition (Wilken et al., 1990). Interestingly, the effect was blocked in the presence of ryanodine, a standard inhibitor of Ca^{++} release (Wier et al., 1985; Karaki et al., 1997) from cardiac sarcoplasmic reticulum (SR) indicating that the mode of cardio-stimulant action of Zo·Cr is related to the function of the SR. Interestingly, gingerols and shogaols have already been reported for their inotropic property via stimulation of SR Ca^{++}-ATPase activity (Kobayashi et al., 1987, 1988; Antipenko et al., 1999) and the observed cardiac stimulatory effect of the ginger extract might be due to the presence of these known components.

The cardio-suppressant effect following the stimulation was resistant to blockade of cholinergic (atropine), adenosine (aminophylline), histamine H_1 receptors (pyrilamine), K^+ channels (glibenclamide) and NO synthesis (L-NAME) suggesting involvement of a possible Ca^{++} antagonist action as evident in the vasodilator effect seen earlier.

The ginger extract was found to contain saponins, terpenoids, flavonoids, amino acids/peptides, secondary amines and alkaloids. We have earlier reported the ability of saponins (Gilani et al., 1994) and flavonoids (Ajay et al., 2003), in other plants, to exhibit hypotensive and vasodilator activities and the presence of such compounds in ginger might possibly contribute in the cardiovascular effects of ginger.

These results show that the aqueous extract of fresh ginger lowers BP via endothelium-dependent (cholinergic) and endothelium-independent (CCB) vasodilator pathways. In atria, an additional cardiotonic component emerged, sensitive to blockade of Ca^{++} release from cardiac SR evident only in the presence of cholinergic receptor blockade. Some of the gingerols tested, showed an atropine-resistant and endothelium-dependent vasodilator effect along with a Ca^{++} antagonist activity that was observed with 6-gingerol. Thus this study justifies the age old use of ginger in hypertension and other cardiovascular disorders and further studies are suggested to identify the chemical(s) responsible for the cholinergic activity.

Acknowledgements

This study was supported by the Pakistan Science Foundation, through a research project # R and D/S-AKU/BIO (178).

References

Ajay, M., Gilani, A.H., Mustafa, M.R., 2003. Effects of flavonoids on vascular smooth muscle of the isolated rat thoracic aorta. Life Sci. 74, 603–612.

Andreopoulos, S., Papapetropoulos, A., 2000. Molecular aspects of soluble guanylyl cyclase regulation. Gen. Pharmacol. 34, 147–157.

Antipenko, A.Y., Spielman, A.I., Kirchberger, M.A., 1999. Interactions of 6-gingerol and ellagic acid with the cardiac sarcoplasmic reticulum Ca^{++}-ATPase. J. Pharmacol. Exp. Ther. 290, 227–234.

Arunlakhshana, O., Schild, H.O., 1959. Some quantitative uses of drug antagonists. Br. J. Pharmacol. 14, 48–58.

Blinks, J.R., 1965. Convenient apparatus for recording contractions of isolated heart muscle. J. Appl. Physiol. 20, 755–757.

Bolton, T.B., 1979. Mechanism of action of transmitters and other substances on smooth muscles. Physiol. Rev. 59, 606–718.

Caulfield, M.P., 1993. Muscarinic receptor: characterization, coupling and function. Pharmacol. Ther. 58, 319–379.

Connell, D.W., McLachlan, R., 1972. Natural pungent compounds: examination of gingerols, shogaols, paradols and related compounds by thin-layer and gas chromatography. J. Chromatogr. 67, 29–35.

Diamond, W.J., 2001. The clinical practice of complementary. Alternative and Western Medicine. CRC Press, Boca Raton, FL, pp. 270–271.

Duke, J.A., 2002. Handbook of Medicinal Herbs. CRC Press, Boca Raton, FL, pp. 327–329.

Furchgott, R.F., Zawadski, J.V., 1980. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 299, 373–376.

Ghayur, M.N., Gilani, A.H., 2005. Ginger lowers blood pressure through blockade of voltage-dependent calcium channels. J. Cardiovasc. Pharmacol. 45, 74–80.

Gilani, A.H., Cobbin, L.B., 1986. Cardioselectivity of himbacine: a muscarinic receptor antagonist. Naunyn-Schmiedeberg's Arch. Pharmacol. 332, 16–20.

Gilani, A.H., Shaheen, F., Saeed, S.A., 1994. Cardiovascular actions of *Daucus carota*. Arch. Pharm. Res. 17, 150–153.

Gilani, A.H., Aziz, N., Ali, S.M., Saeed, M., 2000a. Pharmacological basis for the use of peach leaves in constipation. J. Ethnopharmacol. 73, 87–93.

Gilani, A.H., Aziz, N., Khurram, I.M., Rao, Z.A., Ali, N.K., 2000b. The presence of cholinomimetic and calcium channel antagonist constituents in *Piper betle* Linn. Phytother. Res. 14, 436–442.

Gilani, A.H., Shah, A.J., Ghayur, M.N., Majeed, K., 2005. Pharmacological basis for the use of turmeric in gastrointestinal and respiratory disorders. Life Sci. 76, 3089–3105.

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

Harborne, J.B., 1984. Phytochemical Methods. Chapman and Hall, London, pp. 1–36.

Kapoor, L.D., 1990. Handbook of Ayurvedic Medicinal Plants. CRC Press, Boca Raton, FL, pp. 341–342.

Karaki, H., Ozaki, H., Hori, M., Mitsui-Saito, M., Amano, K., Harada, K., Miyamoto, S., Nakazawa, H., Won, K.J., Sato, K., 1997. Calcium movements, distribution, and functions in smooth muscle. Pharmacol. Rev. 49, 157–230.

Kimura, I., Pancho, L.R., Shiori, T., Kimura, M., 1988. Suppression of spontaneous calcium spikes and contraction in isolated portal veins of mice by gingerols and chemically related compounds. Jpn. J. Pharmacol. 48, 257–262.

Kobayashi, M., Shoji, N., Ohizumi, Y., 1987. Gingerol, a novel cardiotonic agent, activates the Ca^{++}-pumping ATPase in skeletal and cardiac sarcoplasmic reticulum. Biochim. Biophys. Acta 903, 96–102.

Kobayashi, M., Ishida, Y., Shoji, N., Ohizumi, Y., 1988. Cardiotonic action of 8-gingerol, an activator of the Ca^{++}-pumping adenosine triphosphatase of sarcoplasmic reticulum, in guinea pig atrial muscle. J. Pharmacol. Exp. Ther. 246, 667–673.

Langner, E., Greifenberg, S., Gruenwald, J., 1998. Ginger: history and use. Adv. Ther. 15, 25–44.

Mascolo, N., Jain, R., Jain, S.C., Capasso, F., 1989. Ethnopharmacologic investigation of ginger. J. Ethnopharmacol. 27, 129–140.

Mecca, T.E., Love, S.D., 1992. Comparative cardiovascular actions of clentiazem, diltiazem, verapamil, nifedipine, and nimodipine in isolated rabbit tissues. J. Cardiovasc. Pharmacol. 20, 678–682.

NRC, National Research Council, 1996. Guide for the Care and Use of Laboratory Animals. National Academy Press, Washington, D.C., pp. 1–7.

Pancho, L.R., Kimura, I., Unno, R., Kurono, M., Kimura, M., 1989. Reversed effects between crude and processed ginger extracts on PGF2 alpha-induced contraction in mouse mesenteric veins. Jpn. J. Pharmacol. 50, 243–246.

Stahl, E., 1969. Thin Layer Chromatography, 2nd ed. Springer-Verlag, Berlin.

Suekawa, M., Ishige, A., Yuasa, K., Sudo, K., Aburada, M., Hosoya, E., 1984. Pharmacological studies on ginger: I. Pharmacological actions of pungent constituents, 6-gingerol and 6-shogaol. J. Pharmacobio-dyn. 7, 836–848.

Suekawa, M., Aburada, M., Hosoya, E., 1986a. Pharmacological studies on ginger: II. Pressor action of 6-shogaol in anaesthetized rats, or hindquarters, tail and mesenteric beds of rats. J. Pharmacobio-dyn. 9, 842–852.

Suekawa, M., Aburada, M., Hosoya, E., 1986b. Pharmacological studies on ginger: III. Effect of the spinal destruction on 6-shogaol-induced pressor response in rats. J. Pharmacobio-dyn. 9, 853–860.

Thorin, E., Huang, P.L., Fishman, M.C., Bevan, J.A., 1998. Nitric oxide inhibits alpha2-adrenoceptor-mediated endothelium-dependent vasodilation. Circ. Res. 82, 1323–1329.

Tyler, V.E., 1993. The Honest Herbal, 3rd ed. Pharmaceutical Products Press, New York, NY, pp. 147–148.

Varma, K.R., Jain, T.C., Bhattacharyya, S.C., 1962. Structure and stereochemistry of zingiberol and *Juniper camphor*. Tetrahedron 18, 979.

Wagner, H., Bladt, S., Zgainski, E.M., 1984. Plant Drug Analysis. Springer-Verlag, Berlin, pp. 299–304.

Weidner, M.S., Sigwart, K., 2000. The safety of a ginger extract in the rat. J. Ethnopharmacol. 73, 513–520.

Wier, W.G., Yue, D.T., Marban, E., 1985. Effects of ryanodine on intracellular Ca^{++} transients in mammalian cardiac muscle. Fed. Proc. 44, 2989–2993.

Wilken, A., Tawfik-Schlieper, H., Klotz, K.N., Schwabe, U., 1990. Pharmacological characterization of the adenylate cyclase-coupled adenosine receptor in isolated guinea pig atrial myocytes. Mol. Pharmacol. 37, 916–920.

Williamson, E.M., Okpako, D.T., Evans, F.J., 1996. Pharmacological Methods in Phytotherapy Research. Wiley, Chichester, pp. 15–24.