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Muscarinic, Ca^{++} antagonist and specific butyrylcholinesterase inhibitory activity of dried ginger extract might explain its use in dementia

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Abstract

Ginger rhizome *(Zingiber officinale)* has been used for centuries to treat dementia in South Asia. This study was undertaken to possibly justify its use. A 70% aqueous/methanolic extract of dried ginger (Zo.Cr) was used. Zo.Cr tested positive for the presence of terpenoids, flavonoids, secondary amines, phenols, alkaloids and saponins. When tested on isolated rat stomach fundus, Zo.Cr showed a spasmogenic effect (0.03–5.00 mg mL^{-1}); it relaxed the tissue at concentrations $\geq$5 mg mL^{-1}. The stimulant effect was resistant to blockade by hexamethonium and methysergide, but sensitive to atropine, indicating activity via muscarinic receptors. In atropinized (0.1 μM) preparations, Zo.Cr (0.3–3.0 mg mL^{-1}) relaxed high K^+ (80 mM)-induced contractions, indicating Ca^{++} antagonism in addition to the muscarinic effect. This possible Ca^{++} antagonist activity was investigated in Ca^{++}-free conditions, with the inhibitory effect of the extract tested against contractions induced by externally administered Ca^{++}. Zo.Cr (0.1–0.3 mg mL^{-1}), similar to verapamil (0.03–0.10 μM), shifted the contractions induced by externally administered Ca^{++} to the right, thus suggesting an inhibitory interaction between Zo.Cr and voltage-operated Ca^{++} channels. Zo.Cr (0.1–3.0 μg mL^{-1}) also potentiated acetylcholine peak responses in stomach fundus, similar to physostigmine, a cholinesterase inhibitor. Zo.Cr, in an in-vitro assay, showed specific inhibition of butyrylcholinesterase (BuChE) rather than acetylcholinesterase enzyme. Different pure compounds of ginger also showed spasmolytic activity in stomach fundus, with 6-gingerol being the most potent. 6-Gingerol also showed a specific anti-BuChE effect. This study shows a unique combination of muscarinic, possible Ca^{++} antagonist and BuChE inhibitory activities of dried ginger, indicating its benefit in dementia, including Alzheimer's disease.

Introduction

Ginger rhizome (*Zingiber officinale* Roscoe, family Zingiberaceae) is a common food additive, spice and a phytomedicine used since ancient times. It has long been used by traditional healers and as a home remedy for a number of diseases (Kapoor 1990; Gilani & Ghayur 2005). Phytochemical studies have shown that the volatile oil of ginger contains mono- and sesquiterpenes: curcumene, geranyl acetate, terpineol, terpenes, geraniol, α-pinene, limonene, linalool, zingiberene, β-besabolene and α-farnesene, while the pungent principles are gingerol, shogaol, zingerone and paradol (Langner et al 1998; Gilani & Ghayur 2005).

There are two main forms of ginger used: fresh and dried ginger (prepared by drying the fresh rhizome under the sun). Both varieties have their particular uses. For example, fresh ginger is preferred to relieve colds, nausea and rid the body of toxic matter, while dried ginger is reputed to strengthen the stomach, possessing a mild stomach and intestinal stimulant action, and is useful in disorders of the gastrointestinal tract (especially diarrhoea), cough and rheumatism (Foster 2000). Dried ginger has also been traditionally used in dementia and as a memory-enhancing herb (Kapoor 1990; Duke 1995; Khan 2005). Years of research have proven ginger's efficacy in many disorders (Langner et al 1998; Gilani & Ghayur 2005). Our previous work on the different forms of ginger have shown

that dried ginger exhibits anthelmintic properties (Iqbal et al 2006), while fresh ginger possesses gastrointestinal prokinetic, laxative, antidiarrhoeal (Ghayur & Gilani 2005a, 2006; Ghayur et al 2007), tocolytic (Ghayur & Gilani 2007), bronchodilator, airway relaxant (Ghayur & Gilani 2007; Ghayur et al 2008), hypotensive, vasodilator and cardiosuppressant properties (Ghayur & Gilani 2005b; Ghayur et al 2005). Continuing our endeavours to further unravel the medicinal properties of ginger, we report here that the 70% aqueous/methanolic extract of dried ginger exhibits muscarinic, possible Ca^{++} antagonist and cholinesterase inhibitory activities. This study reiterates some of the activities that we have already seen with the fresh ginger extract in earlier studies, for example the gastric stimulant and relaxant activities (Ghayur & Gilani 2005a, 2006; Ghayur et al 2007). Furthermore, this study shows for the first time that ginger possesses cholinesterase inhibitory potential, which, together with its muscarinic and Ca^{++} antagonist activities, highlights the possible usefulness of ginger in dementia. Drug therapy for Alzheimer's disease (AD) has primarily concentrated on the cholinergic system as AD progression is accompanied by a loss of central cholinergic neurons (Muir 1997). In order to revitalize cholinergic function, approaches have involved stimulating cholinergic receptors with muscarinic agonists or potentiating the availability of acetylcholine (ACh) by inhibiting cholinesterase enzymes (Howes & Houghton 2003). Several muscarinic agonists have been reported in the literature for their ability to restore cholinergic function and attenuate cognitive function seen in AD (Langmead et al 2008).

Materials and Methods

Drugs and standards

The following reference chemicals were obtained from Sigma Chemical Company (St Louis, MO, USA): ACh chloride, atropine sulfate, carbachol (CCh) chloride, hexamethonium chloride, methysergide maleate, physostigmine hydrochloride, serotonin (5-HT) hydrochloride and verapamil hydrochloride. The following chemicals were used to make the physiological salt solutions: potassium chloride (Sigma Chemical Company), calcium chloride, glucose, magnesium sulfate, potassium dihydrogen phosphate, sodium bicarbonate and sodium chloride (E. Merck, Darmstadt, Germany) and ethylenediaminetetra-acetic acid (EDTA) from BDH Laboratory Supplies (Poole, England). The commercially available ginger pure compounds namely, 6-, 8- and 10-gingerol and 6-shogaol were obtained from Chromadex (Irvine, CA, USA). Stock solutions of all the chemicals were made in saline and the dilutions were made fresh on the day of the experiment.

Animals

The 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 Ethics Review Committee of Aga Khan University. Sprague–Dawley rats (170–200 g) of either sex were housed in the animal house of Aga Khan University under a controlled environment (23–25°C). They were fed a standard diet consisting of (g kg^{-1}): flour 380, fibre 380, molasses 12, NaCl 5.8, nutrivet L 2.5, potassium metabisulfate 1.2, vegetable oil 38, fish meal 170 and powdered milk 150. The animals were fasted for 24 h before the experiment but were given free access to tap water.

Plant material and extract preparation

A total of 1021 g of dried ginger rhizome was bought from a wholesale market in Karachi, Pakistan. Ginger rhizomes were sliced to expose the inner part, soaked in 8 L of 70% aqueous/methanol and kept for a total of 3 days. After 3 days, the extract was filtered through a porous cloth and the filtrate collected while the plant material was again soaked in 8 L of solvent for 3 days, twice. The combined filtrate was filtered through Whatman qualitative grade-1 filter paper and later concentrated in a rotary evaporator to obtain a thick brown extract (Zo.Cr; 129 g) with a yield of 12.6% (w/w).

Preliminary phytochemical analysis

The crude extract (Zo.Cr) was screened for the presence of different classes of compounds by thin-layer chromatography using silica gel G (E. Merck) plates of 0.25-mm thickness (Wagner et al 1984; Gilani et al 2006). The extract was dissolved in chloroform and methanol (2:1), while the development of plates was carried out with chloroform and 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); sulfuric acid and heating at 105°C for 5 min (organic compounds); 0.5% anisaldehyde in sulfuric 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); Dragendorff 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.

Isolated rat stomach fundus

Rats were killed by cervical dislocation (Ghayur & Gilani 2005a; Ghayur et al 2007). The stomach was removed and placed in Kreb's solution for isolating the fundus. The stomach was opened along the lesser curvature and divided into two longitudinal strips, 2 mm wide and 15 mm long. Each strip preparation was mounted separately in a 10-mL

tissue bath with Kreb's solution at 37°C and aerated with carbogen (5% CO_2 in O_2). The composition of Kreb's solution was (mM): 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). Basal tension of 1 g was applied to each tissue and the responses recorded following an equilibrium period of 60 min. Submaximal concentrations of CCh (0.3 μM) were tested repeatedly to stabilize the preparation and the responses were recorded through isotonic Harvard transducers coupled with Harvard student oscillographs.

Experimental protocol

The ginger crude extract and its pure compounds were tested first on the resting baseline of fundic preparations. A possible stimulant activity was compared with that of standard CCh and serotonin. To determine the mode of action of this stimulant effect, the test substance response was challenged with antagonists such as hexamethonium (0.3 mM), atropine (0.1 μM) and methysergide (0.1 μM). Later, the extract and pure compounds were also tested for a possible relaxant activity. To assess whether the spasmolytic activity of the ginger extract and its pure compounds was mediated through Ca^{++}-channel blockade (CCB), high K^+ (80 mM) was used to depolarize the fundic preparations (Farre et al 1991) and produce a sustained contraction. Samples were then added in a cumulative fashion to obtain concentration-dependent inhibitory responses (van-Rossum 1963). The relaxation of tissue preparations precontracted with high K^+ was expressed as a percentage of the control response mediated by high K^+. Contractions of smooth muscles induced by high K^+ (>30 mM) are known to be mediated via influx of Ca^{++} through voltage-operated Ca^{++} channels (VOCC) from extracellular fluid. A substance that inhibits these contractions might possibly act through CCB (Bolton 1979).

To further investigate possible CCB activity of the extract, the tissue preparation 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 all the Ca^{++} from the tissue to achieve a Ca^{++}-free environment. 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, contractions were induced with externally administered Ca^{++}. These externally administered Ca^{++} contractions were reproduced and when found superimposable (usually after two cycles), the tissue was pretreated with increasing concentrations of extract and positive control (separately) for 60 min to test for a possible CCB effect. The externally administered Ca^{++}-induced contractions were reconstructed in the presence of different concentrations of Zo.Cr, while verapamil, a standard CCB (Bolton 1979; Farre et al 1991), was used as a positive control.

To screen the extract for potential cholinesterase inhibitory activity in the fundus preparations, maximal peak responses of ACh (1 μM) were reproduced. Later, these control peak responses were pretreated for 30 min with increasing concentrations of Zo.Cr or physostigmine, a standard cholinesterase inhibitor (Robinson 1968). Any enhancement in the control response of ACh, after pretreatment with extract or standard for 30 min, was possibly due to inhibition of cholinesterase enzymes, which resulted in an augmented ACh response (Gilani et al 2004, 2005). Further confirmatory tests for cholinesterase inhibitory activity were performed using an enzyme inhibition assay as detailed below.

Enzyme assay for cholinesterase inhibition

Acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibitory activities of Zo.Cr and pure compound were measured in-vitro by a modified spectrophotometric method developed by Ellman et al (1961). Electric eel AChE (type VI-S; Sigma Chemical Company), and horse serum BuChE (Sigma) were used as the enzyme source, while acetylthiocholine (ATCh) iodide and butyrylthiocholine (BTCh) chloride (Sigma) were used as substrates in the respective enzyme assays. Ellman reagent (i.e. 5,5-dithiobis (2-nitro)benzoic acid or DTNB; Sigma) was used to develop the chromogenic marker for the measurement of the cholinesterase activity. Sodium phosphate buffers (1 mM) at pH 7.0 and 8.0 were used to prepare the enzyme working solution and in the assay mixture, respectively. Concentrated enzyme preparations were prepared and stored at −70°C, and diluted at the time of the experiments (the activity of the enzyme was not affected under these conditions for several months).

All the inhibition studies were performed in 96-well microtitre plates. For the assay procedure, 140 μL of 0.1 mM sodium phosphate buffer (pH 8.0), 20 μL of test compound solution, and 20 μL of AChE/BuChE solution were mixed and incubated for 15 min at 25°C. Then, 10 μL of DTNB was added and the reaction was then initiated with the addition of 10 μL of ATCh/BTCh (0.71 and 0.2 mM of ATCh and BTCh, respectively). The hydrolysis of ATCh and BTCh was monitored by measuring the formation of the yellow 5-thio-2-nitrobenzoate anion as a result of the reaction of DTNB with thiocholine at a wavelength of 412 nm using a SpectraMax microplate spectrophotometer (Molecular Devices, CA, USA). Test substances were dissolved in 5% ethanol, while the control received only the same volume of the solvent. All reactions were performed in triplicate and the initial rate was measured as the rate of change in optical density (OD) min^{-1} and used in subsequent calculations. According to Ellman et al (1961), since the extinction coefficient of the yellow anion is known, the rate of the enzymatic reaction can be calculated based on the following equation:

$$\text{Rate (mol L}^{-1}\text{ min}^{-1}\text{)} = \text{change in absorbance min}^{-1}/13\,600.$$

The percentage enzyme inhibition by the test sample was calculated using the following formula:

$$\%\ \text{Inhibition} = 100 - (\text{change in absorbane of test/change in absorbance of control} \times 100)$$

The test reaction was the enzymatic reaction containing the test sample, while the control was the enzymatic reaction lacking the test sample. Physostigmine was used as a standard cholinesterase inhibitor (Robinson 1968).

Statistical analysis

All the data are expressed as mean ± s.e.m. (n is the number of experiments) and the median effective concentrations (EC50 values) with 95% confidence intervals. The unpaired Student's *t*-test, one-way and two-way analysis of variance were used to compare mean values, while for median values the Kruskal–Wallis test was used (GraphPAD software; GraphPAD, San Diego, CA, USA). A probability of less than 0.05 was considered statistically significant. Concentration–response curves were analysed by non-linear regression (GraphPAD software).

Results

Phytochemical analysis

Zo.Cr showed the presence of lipophilic and organic compounds along with the following classes of compounds: terpenoids, flavonoids, amino acids/peptides, secondary amines, phenols, alkaloids and saponins. Coumarins and anthraquinones were absent in the extract.

Effect of extract on resting baseline of rat stomach fundus

Zo.Cr produced a concentration-dependent (0.03–5.00 mg mL^{-1}) contractile effect (Figure 1A) on the resting baseline of rat stomach fundus, with an EC50 value of 0.26 mg mL^{-1} (0.17–0.40, n = 5). The efficacy of the spasmogenic effect was 86.8 ± 2.8% of the CCh maximum effect (Figure 1A). An extract concentration of ≥5 mg mL^{-1} showed relaxation of the tissue. Pretreatment of the tissue with atropine (0.1 μM), but not with hexamethonium (0.3 mM) or methysergide (0.1 μM), completely blocked the effect of Zo.Cr. The effect of CCh was also blocked by atropine.

Spasmolytic effect of extract on rat stomach fundus

In the atropinized (0.1 μM) tissue preparation, Zo.Cr inhibited high K^+-induced contractions in a concentration-dependent manner (0.3–3.0 mg mL^{-1}) with an EC50 value of 0.81 mg mL^{-1} (0.73–0.89, n = 5; Figure 1B). In order to further investigate this relaxant effect of the extract, contractions were induced with externally administered Ca^{++} (in an initial Ca^{++}-free environment) and these Ca^{++} contractions were pretreated with increasing concentrations of extract. Zo.Cr (0.1–0.3 mg mL^{-1}, n = 4) shifted these Ca^{++}-induced contractions to the right (Figure 2A), similar to the effect exhibited by verapamil (0.03–0.10 μM, n = 4; Figure 2B).

Preliminary screening of the extract for a cholinesterase inhibitory effect in rat stomach fundus

In the presence of increasing concentrations of the extract (0.0001–0.0030 mg mL^{-1}, n = 3–12), the stimulant effect of a fixed dose of ACh (1 μM) was enhanced in a concentration-dependent fashion (Figure 3A). Similarly, physostigmine (0.01–0.10 μM, n = 5–7) also potentiated the ACh peak response (Figure 3B), indicating similarity in the modes of action.

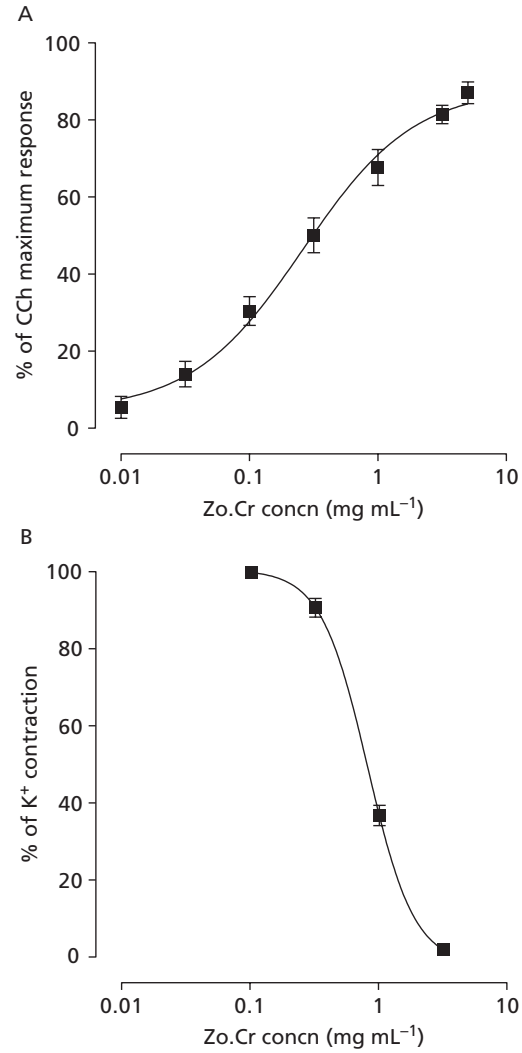

Figure 1 Concentration–response curves showing the effect of dried ginger crude extract (Zo.Cr) on resting baseline (A) and high K^+ (80 mM) contracted (B) isolated rat stomach fundus tissues. The spasmogenic effect was calculated in comparison with the carbachol (CCh) maximum response, while the spasmolytic effect was calculated in comparison with the % of K^+ contraction. Values shown are mean ± s.e.m., n = 5. There was a significant difference between the individual extract concentrations in both the curves ($P < 0.0001$; one-way analysis of variance).

Effect of extract on in-vitro cholinesterase enzyme inhibition assay

In the in-vitro assay, the extract was tested for a potential inhibitory effect on both BuChE and AChE enzymes. It was observed that Zo.Cr concentration-dependently (0.0625–1.0000 mg mL^{-1}, n = 3) inhibited BuChE, with an EC50 value of 0.18 mg mL^{-1} (0.17–0.19, n = 3). Maximum BuChE enzyme inhibition produced by the extract was 71.9 ± 0.6% at a concentration of 1 mg mL^{-1}. Compared with the maximum inhibition shown against BuChE, the extract (1 mg mL^{-1}) showed only minor inhibition of AChE (32.2 ± 1.7%; $P < 0.0001$). Physostigmine, the standard cholinesterase inhibitor, inhibited both BuChE and AChE, with EC50 values

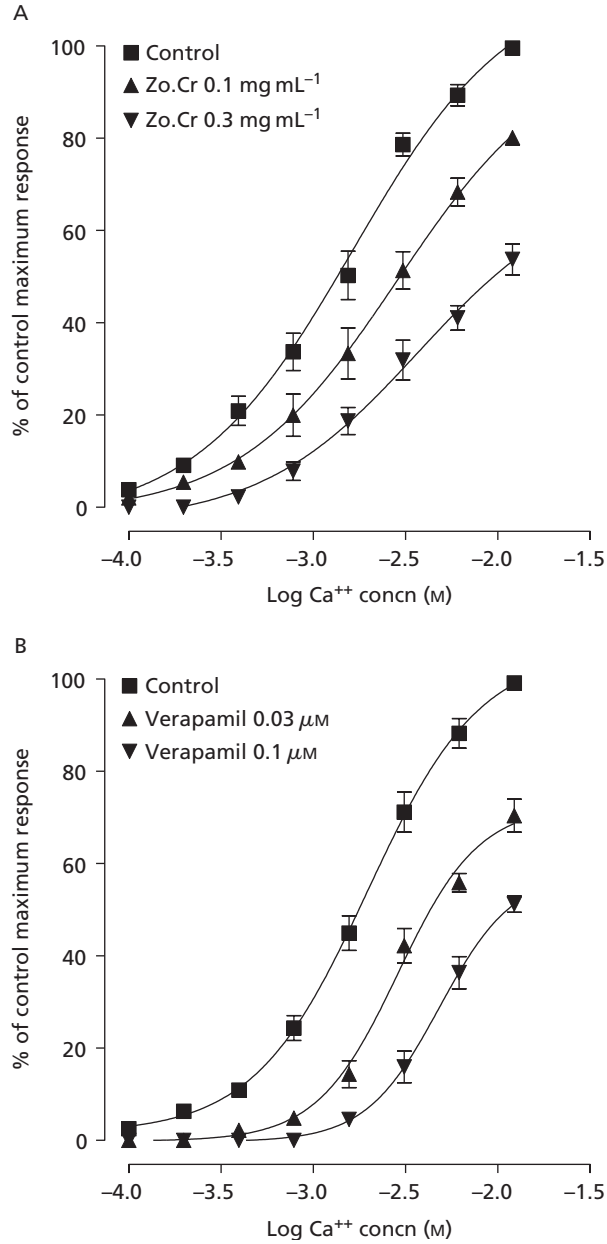

Figure 2 Concentration–response curves showing the effect of increasing concentrations of dried ginger crude extract (Zo.Cr) (A) and verapamil (B), on Ca^{++} concentration–response curves constructed in a Ca^{++}-free medium in isolated rat stomach fundus. Values shown are mean ± s.e.m., n = 4. There was a significant difference between Zo.Cr and verapamil treatments compared with control curves ($P < 0.0001$) and between individual concentrations in all curves ($P < 0.0001$; two-way analysis of variance).

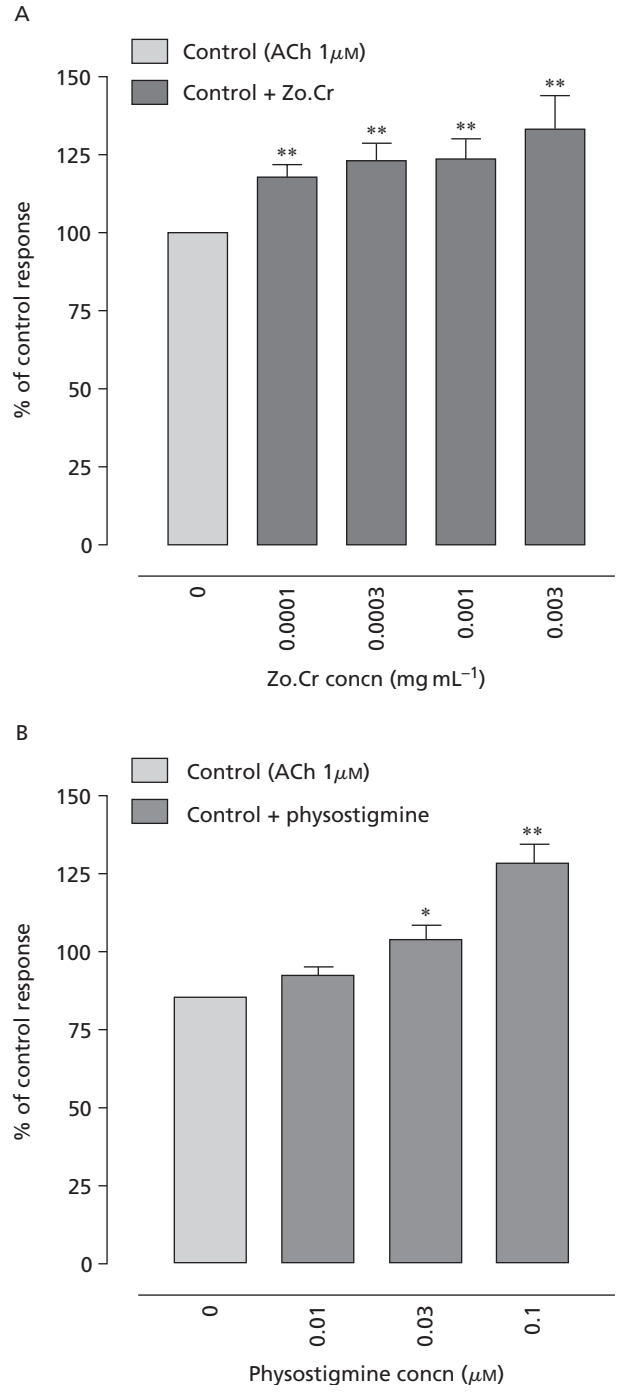

Figure 3 Bar diagrams showing the effect of increasing concentrations of dried ginger crude extract (Zo.Cr) (A) and physostigmine (B) on control acetylcholine (ACh, 1 μM) responses in rat stomach fundus tissues. Values shown are mean ± s.e.m. n = 3–12. *$P < 0.05$ and **$P < 0.01$ compared with the ACh control response (one-way analysis of variance followed by Dunnett's test).

of 0.85 μM (0.83–0.89, n = 4) and 0.04 μM (0.03–0.06, n = 4), respectively.

Effect of ginger pure compounds on rat stomach fundus and cholinesterase assay

When tested on the resting baseline of rat stomach fundus, the ginger pure compounds, namely 6-gingerol, 8-gingerol, 10-gingerol and 6-shogaol (Figure 4), showed no effect up to the tested concentration of 1 mM. Anticipating some relaxant activity, the compounds were then administered against high K^+-induced contractions. All of the compounds (6-gingerol,

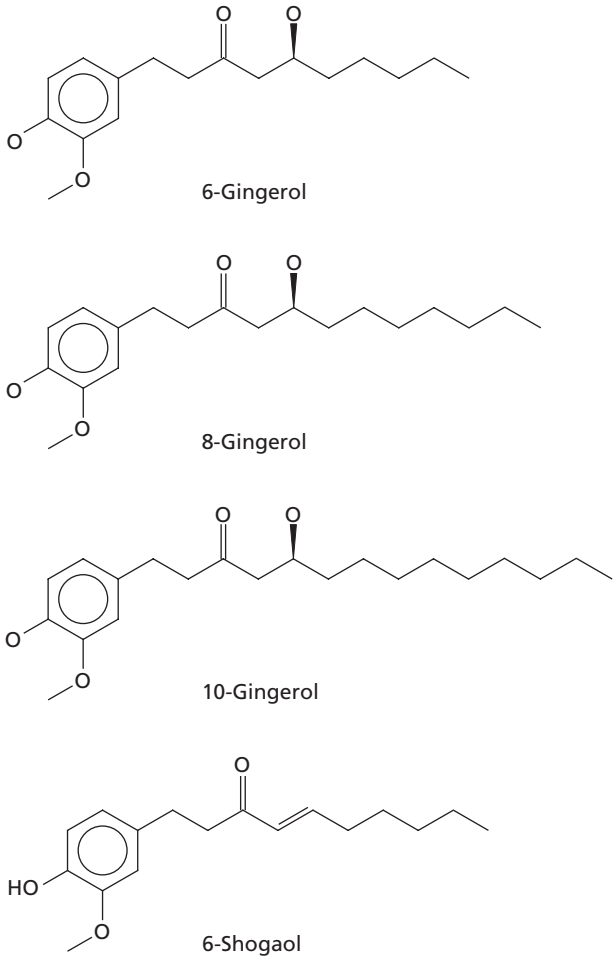

Figure 4 Chemical structures of the standard ginger compounds 6-, 8- and 10-gingerol and 6-shogaol.

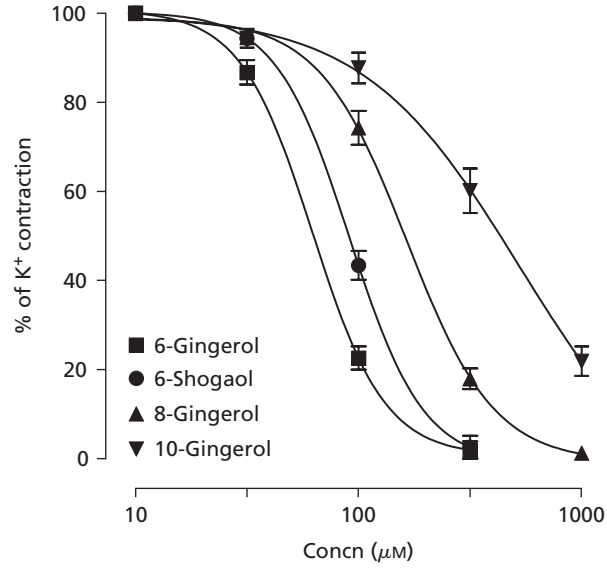

Figure 5 Concentration–response curves showing the relaxant effect of increasing concentrations of ginger compounds, 6-, 8- and 10-gingerol and 6-shogaol, on high K^+ (80 mM) induced contractions in isolated rat stomach fundic preparations. Values shown are mean ± s.e.m., n = 3. There was a significant difference between all the curves ($P < 0.0001$) and between individual concentrations in all the curves ($P < 0.0001$; two-way analysis of variance).

8-gingerol, 10-gingerol and 6-shogaol) showed concentration-dependent (30–1000 μM) relaxant activity (Figure 5), with EC50 values of 62.2 μM (52.9–73.1, n = 3), 164.2 μM (142.0–189.9, n = 3), 528.4 μM (112.6–2480.0, n = 3) and 91.1 μM (80.6–103.1, n = 3), respectively. Although the EC50 values do not indicate any difference ($P > 0.05$), Figure 5 shows that the compound 6-gingerol was significantly more potent in mediating the relaxant effect than the other compounds.

Owing to insufficient quantity of pure compounds, we could only screen the most potent compound, 6-gingerol, for in-vitro cholinesterase inhibitory activity. The compound was tested against the cholinesterase enzymes, AChE and BuChE. It was observed that the compound was inactive against AChE when tested up to a concentration of 1 mM. For BuChE, 6-gingerol showed a concentration-dependent (0.5–1.0 mM) inhibitory effect, with an EC50 value of 0.89 mM (0.76–1.03, n = 3). The maximum inhibitory effect produced by the compound for BuChE was 54.0 ± 1.5% with concentration of 1 mM.

Discussion

This study was performed to rationalize and investigate the use of dried ginger in dementia (Kapoor 1990; Duke 1995; Khan 2005). The aqueous/methanolic extract of dried ginger showed a concentration-dependent contractile effect on the resting baseline of rat stomach fundus tissue preparations. This stimulant activity was resistant to blockade by methysergide, a non-selective serotonin antagonist (van Zwieten et al 1990) and hexamethonium, a ganglion blocker (Klowden et al 1978). Rat stomach fundus is known to have serotonin receptors that mediate contractility (Komada & Yano 2007). The stimulant effect of Zo.Cr was completely abolished in the presence of atropine, a muscarinic receptor blocker (Arunlakhshana & Schild 1959). Atropine is known to block the effect (not receptors) of nicotine, as the end effect of nicotine in gastrointestinal tract is ultimately due to release of ACh, which as a result acts on muscarinic receptors (Brown & Taylor 1995). The observation that the stimulant effect of Zo.Cr was insensitive to hexamethonium but sensitive to atropine indicates that the spasmogenic effect was mediated through stimulation of muscarinic receptors. The presence of muscarinic receptors has been reported in rat stomach fundus (Ghayur et al 2007) mediating contractile responses, with the muscarinic M_1 and M_3 subtypes being most important (Milovanović & Janković 1997; Smaili et al 1997).

In addition to the ACh-like spasmogenic effect, Zo.Cr also exhibited a spasmolytic effect in fundus. The extract, for its spasmolytic effect, not only relaxed the high K^+

(80 mM)-induced contractions in the tissues, but also inhibited contractions that were induced, in initial Ca^{++}-free conditions, with externally administered Ca^{++}. This activity is a typical characteristic of Ca^{++} antagonists (Furchgott 1961). Verapamil, a standard CCB (Bolton 1979; Farre et al 1991), also exhibited similar results. Although the results suggest interaction of the extract with VOCC, this cannot be confirmed until more intensive studies are performed on Ca^{++} currents at the level of single smooth muscle cells. If the possible presence of Ca^{++} antagonistic activity is confirmed in the extract, then this would be of special interest as CCBs have been found to be useful in preventing dementia and AD. This is mainly due to the role played by Ca^{++} in regulating brain functions (Vagnucci & Li 2003). Ca^{++} links membrane excitation to subsequent intracellular enzymatic response and the change in Ca^{++} homeostasis is linked to ageing, with consequences on higher cortical functions (Arrieta & Birks 2001). Cytosolic $[Ca^{++}]_i$ also increases with ageing, mediated via neuronal cell body VOCC. This activates an apoptotic gene that results in cholinergic neuronal death (Branconnier et al 1992). CCBs can thus have a palliative effect on progression and prevention of AD.

Preliminary screening of the extract for a possible cholinesterase inhibitory effect was done in rat stomach fundus. Fixed concentrations of ACh (1 μM) were pretreated with increasing concentrations of Zo.Cr. The extract potentiated ACh peak responses, similar to physostigmine, a standard cholinesterase inhibitor (Robinson 1968), thus indicating similarity in the modes of action. The cholinesterase inhibitory effect of Zo.Cr was confirmed in the in-vitro cholinesterase assay when, similar to physostigmine, it inhibited both BuChE and AChE enzymes. Interestingly, the extract was significantly more potent in inhibiting BuChE than AChE enzymes ($P < 0.0001$). Cholinesterase enzymes bind and cleave ACh to choline and acetate. Inhibitors of cholinesterase produce a cholinergic action by preventing hydrolysis of ACh formed endogenously at the cholinergic nerve endings (Mycek et al 1997) and are considered useful in AD (Palmer 2002). This BuChE-specific inhibitory effect of the extract is of particular interest given the recent interest in AD therapy focusing on the benefits of specific BuChE inhibitors (e.g. cymserine and MF-8622) and dual BuChE/AChE inhibitors (e.g. rivastigmine) over the well known specific AChE inhibitors (e.g. donepezil and galantamine) (Greig et al 2002; Mesulam et al 2002; Rösler 2002). In healthy brains, AChE is known to be the main player (80% workload), compared with BuChE, when it comes to hydrolysis of ACh (Greig et al 2001). Recent research has shown that in patients with severe AD, BuChE activity increases, while that of AChE either stays stable or declines (by as much as 90%), indicating the importance of targeting BuChE in severely diseased AD patients (Giacobini 2003; Greig et al 2001).

In order to trace some of the activities seen with the extract, we acquired some commercially available known phenolic compounds of ginger (Gilani & Ghayur 2005a, b; Langner et al 1998). Three different gingerols (6-, 8- and 10-gingerol) and one shogaol (6-shogaol) were tested on isolated fundus. For reference, we performed a chemical analysis of the extract and found that the extract did contain phenolic compounds. Unlike Zo.Cr, none of the tested compounds showed any spasmogenic activity, but all exhibited a spasmolytic effect on high K^+-contracted rat fundus, thus indicating activity via blockade of VOCC. 6-Gingerol was the most potent compared with the other compounds ($P < 0.0001$). Gingerols and shogaols are the main components of ginger. If these compounds do not possess the ACh-like spasmogenic activity it might be due to the many other compounds present in ginger as shown by the chemical analysis. Among the gingerols, the spasmolytic activity decreased with increasing size of the side chain in the chemical structure (Figure 4). Both 6-gingerol and 6-shogaol have the same number of C atoms in their side chains, however the former, which lacks an extra hydroxyl group and a double bond in the side chain, was more potent than the latter. Due to experimental limitations, we cannot stipulate the concentrations of these pure compounds in the extract. However, in a recent study, Lee et al (2007) did a very similar quantification. They reported that these same gingerols (6-, 8- and 10-gingerol) and 6-shogaol have a concentration of around 2–9 mg g^{-1} of the raw dried herb, while the amounts were 5-times lower in the dried herb extract.

Owing to the limited quantity of the compounds, we could only test 6-gingerol for anticholinesterase activity in the in-vitro assay. Similar to the extract, 6-gingerol also showed a BuChE-specific inhibitory effect, with no activity against AChE. Thus, we have identified a possible specific BuChE-inhibiting compound, with CCB potential, from a natural source. We have in the past identified a number of pure compounds with dual cholinesterase and CCB activities (Khalid et al 2004; Choudhary et al 2005a, b; Atta-ur-Rahman et al 2006). Related to our findings, there have been recent advances in the development of hybrid molecules/designer compounds with the ability to target multiple sites of actions in the treatment of AD. This may include cholinesterase inhibitory activity along with some particular receptor blocking activity, antioxidant activity or even CCB activity (Decker 2007).

The concentrations at which the extract exhibited the muscarinic, possible Ca^{++} antagonistic and cholinesterase inhibitory activities are comparable with the concentrations of other extracts reported in the literature. We did not perform any in-vivo tests; however, comparing the results seen here with some previously published results (from studies with in-vivo data) of fresh ginger extract (Ghayur & Gilani 2005a, 2006), we can say that dried ginger extract mediates its effects at concentrations that correlate with the 1–2 g of raw dried ginger that is usually taken by people to get the required relief.

In the past, different studies have shown that ginger possesses anxiolytic properties (Hasenöhrl et al 1996), improves inhibitory avoidance learning (Topic et al 2002a), facilitates spatial learning along with reducing oxidative stress (Topic et al 2002b) and inhibits β-amyloid peptide-induced cytokine and chemokine expression in monocytes, thus delaying the onset and progression of neurodegenerative disorders (Grzanna et al 2004). All the studies mentioned

above, together with our findings, suggest a possible benefit of ginger in memory disorders such as AD.

Conclusion

The results show that dried ginger extract possesses muscarinic, Ca^{++} antagonist and specific BuChE inhibitory properties. The gingerols and 6-shogaol all showed spasmolytic activities possibly mediated via Ca^{++} antagonism, while 6-gingerol also exhibited BuChE-specific inhibitory activity in the in-vitro cholinesterase inhibition assay. These results give support to the traditional use of ginger in dementia. The results of this study are only preliminary and further studies are necessary to determine the mechanism of action of this herb at the receptor (muscarinic), channel (Ca^{++}), enzyme (cholinesterase) and signal pathway levels. Ultimately, only clinical studies can determine the overall safety and efficacy of the ginger extract, including its ability to cross the blood–brain barrier (as only then can it be of any use in memory disorders).

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