

Spasmolytic effect of citral and extracts of *Cymbopogon citratus* on isolated rabbit ileum*

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Abstract

Cymbopogon citratus, commonly known as lemongrass, has been shown to have antioxidant, antimicrobial and chemo-protective properties. Citral, a monoterpenoid, is the major constituent of *C. citratus* that gives off a lemony scent and is postulated to be responsible for most of its actions. In addition, *C. citratus* has been traditionally used to treat gastrointestinal discomforts, however, the scientific evidence for this is still lacking. Thus, the aim of the present study was to investigate the effect of the extracts of various parts of *C. citratus* (leaves, stems and roots) and citral on the visceral smooth muscle activity of rabbit ileum. The effect of the test substances were tested on the spontaneous contraction, acetylcholine (ACh)- and KCl-induced contractions. Citral at doses between 0.061 mM to 15.6 mM and the extract of leaves at doses between 0.001 mg/mL to 1 mg/mL significantly reduced the spontaneous, ACh- and KCl-induced ileal contractions. When the ileum was incubated in K⁺-rich-Ca²⁺-free Tyrode's solution, it showed only minute contractions. However, the strength of contraction was increased with the addition of increasing concentrations of CaCl₂. The presence of citral almost abolished the effect of adding CaCl₂, while the leaf extract shifted the calcium concentration-response curve to the right, suggesting a calcium antagonistic effect. These results were similar to that elicited by verapamil, a known calcium channel blocker. In addition, the spasmolytic effect of citral was observed to be reduced by the nitric oxide synthase inhibitor, L-NAME. In conclusion, citral and the leaf extract of *C. citratus* exhibited spasmolytic activity and it appeared that they may act as calcium antagonists. Furthermore, the relaxant effect of citral, but not that of the leaf extract may be mediated by nitric oxide suggesting the presence of other chemical components in the leaf extract other than citral.

Key words: *Cymbopogon citratus*, citral, spasmolytic, isolated rabbit ileum

Introduction

The use of plants in modern pharmacotherapy involves the isolation of active compounds

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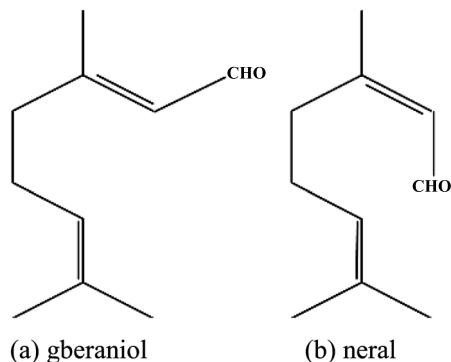


Fig. 1. Chemical structure of citral (a) geraniol (*trans*-isomer), (b) neral (*cis*- isomer).

which requires prior extensive scientific investigations on their biochemical, physiological and pharmacological effects. Generally, the major components are found to reflect well the biophysical as well as the biological features of the medicinal plant. Citral, 3-7-dimethyl-2, 6-octadienal, is the major component of *Cymbopogon citratus* (Yang *et al.*, 2009) and has been reported to be the most important member of the open chain monoterpenoids (AL-Shaer, 2006). Chemically, citral is a mixture of isomers, *trans*-isomer geraniol (40 to 62%) and *cis*-isomer neral (25 to 38%) with the same molecular formula, but having different structures (Fig. 1).

C. citratus (lemongrass) is a tropical perennial herb and is native to India and Sri Lanka (Carlini *et al.*, 1986). It is also thought to have its origin in Malaysia and can be found growing in most parts of South East Asia (Carlini *et al.*, 1986). It falls into the grass family of Poaceae. In Malaysia *C. citratus* is known as 'serai makan' or edible lemongrass (Muhammad and Ali, 1994) because it is extensively used in the local cuisine due to its lemony flavour. It is also consumed as an aromatic drink to ease various intestinal discomforts such as poor digestion, stomach aches and bowel spasms.

C. citratus is used throughout many regions of the world as a medicinal plant. It has been claimed to be effective in reducing fever, infection, rheumatism, headaches and abdominal discomfort, as well as acting as sedative, analgesic, anti-inflammatory, antispasmodic, diuretic, carminative and antihypertensive agents (Borrelli and Izzo, 2000; Runnie *et al.*, 2004).

Studies on an aqueous suspension of citral in mouse micronucleus test system showed it to have an anti-clastogenic effect (Rabbani *et al.*, 2006). In addition, citral, at a concentration comparable to that found in a cup of tea brewed with 1 g lemongrass, was found to induce apoptosis in several haematopoietic cancer cell lines (Dudai *et al.*, 2005). Although there have been many scientific studies on the various medicinal effects of lemongrass, the scientific reports regarding its effects on the smooth muscle of the gastrointestinal tract are still lacking.

The objectives of the present study were therefore, to examine the effects of citral and extracts from leaves, stems and roots of *C. citratus* on the contraction of rabbit ileum and to determine their mechanisms of actions.

Materials and Methods

Drugs

Citral, acetylcholine chloride (ACh), atropine sulphate salt hydrate, N^o-nitro-L-arginine methyl ester hydrochloride (L-NAME), verapamil hydrochloride, ethylenediaminetetraacetic acid disodium salt (EDTA) were purchased from Sigma Chemical Co. (USA). With the exception of citral, a stock solution of each drug was prepared and diluted to the desired concentrations in distilled water. Meanwhile 2% methanol was used as a vehicle for citral. All drugs were prepared fresh on the day of the experiment. The concentrations of the drugs were expressed as the final molar concentration contained in the tissue bath. The respective vehicles of the test substances neither affected the spontaneous motility nor modified the response to the test materials under investigation. Chemicals used for making Tyrode's solution were NaCl (8.0 g), KCl (0.2 g), CaCl₂ (0.2 g), MgCl₂ (0.1 g), NaHCO₃ (1.0 g), NaH₂PO₄ (0.05 g) and glucose (1.0 g) which was made up to 1000 mL in distilled water. The pH was subsequently adjusted to 7.4. In experiments that required calcium-free Tyrode's, the CaCl₂ was replaced with 0.1 mM EDTA as described by Ghayur and Gilani (2005), Gilani *et al.* (2005d) and Estrada-Soto (2007).

Plant material and preparation of methanolic extracts of *Cymbopogon citratus*

C. citratus was collected from the rural area of Beranang, Kajang, Selangor, and identified by the *Rimba Ilmu*, Botanical Department, Faculty of Science, University of Malaya (voucher identification code: KLU 045309). The procedures for preparation of the plant extracts were as described by Ghayur and Gilani (2005). The *C. citratus* was cleaned and isolated into three different parts namely, leaves, stems and roots. They were oven dried at 65°C and thereafter, coarsely ground. The ground materials of *C. citratus* (100 g) were extracted with 70% methanol for 3 days with occasional shaking. The extracts were then put onto a rotary evaporator under reduced pressure (-760 mmHg) at 40°C to eliminate the methanol. Extracts were subsequently lyophilized and 12.34 g of leaves, 14.38 g of stems and 11.68 g of roots were obtained. The extracts were labelled as LE for leaf extract, SE for the stems and RE for the roots, and stored at -20°C until pharmacological investigations were performed. All the extracts were prepared and diluted in distilled water to the requirements of the studies and a volume of not more than 1,000 µL was added into a 10 mL tissue bath.

Citral

Citral was diluted in 2% (*v/v*) methanol to make it more aqueous. Various dilutions of citral was then prepared in 2% methanol according to the requirements of the studies (Subramanian *et al.*, 2002). A volume of 100 µL of the dosing solution was added into a 10 mL tissue bath.

Animals

Adult male New Zealand White Rabbits weighing 2.0 to 2.5 kg were obtained either from the University of Malaya or the National University of Malaysia Laboratory Animal Centres. The rabbits were housed in a standard experimental animal room. They were given tap water and standard diet, *ad libitum*, but the food was withdrawn 24 hours prior to the experiments. The

experimental protocol reported in this manuscript was approved by the Animal Care and Use Committee of the Faculty of Medicine, University of Malaya (ACUC: Reference number-FIS/07/12/2006/RI(R)).

In vitro biological activity

Male New Zealand White rabbits were sacrificed by a blow on the back of the head. The abdomen was then opened. The caecum was lifted forward and the ileum identified. The ileum was subsequently dissected out and placed in fresh ice-cold Tyrode's solution. The mesenteries were cleaned and the lumen was washed with Tyrode's solution to remove its contents. A threaded needle was passed through the ileum wall from one cut end and looped. The same procedure was repeated on the other end of the ileum (Jain, 2000). The strips of whole ileum, approximately 1.5 to 2.0 cm long were then suspended individually in a 10 ml tissue bath containing Tyrode's solution, with one end anchored at the bottom of the organ bath while the other end was connected to a force transducer. The organ bath was maintained at 37°C and aerated with a mixture of 95% O₂ and 5% CO₂. The changes in the force generated by the muscle contraction were determined by an isometric force transducer (MLT050/D, ADI instrument) connected to the PowerLab recording device (LabChart Pro 6.0.-ADI Instrument, Australia) coupled with a portable computer display monitor. An initial tension load of 1 g was applied to the ileum and the intestinal responses were recorded. Each piece of ileum was allowed to equilibrate and stabilise for at least 45 min with washout every 10 min, before addition of any drug or test material. Since the whole ileum was used, the preparations did not separate out the actions of the longitudinal muscle from circular muscle and the recording obtained was the result of the activity of both muscles of the ileum. The strength of the contraction was determined from the amplitude of the rhythmic contraction while the frequency of the muscle contraction was obtained by counting the number of peaks per unit time and the changes observed before and after addition of test materials were analysed accordingly.

Pharmacological test

Effect of citral and the methanolic extracts of C. citratus (LE, SE and RE) on the spontaneous contraction

Since rabbit ileum exhibited spontaneous rhythmic contractions, the relaxant effects of the test materials were able to be tested directly without the use of an agonist. Citral at concentrations between 0.061 mM to 15.6 mM, the extracts at doses between 0.001 mg/mL to 1 mg/mL and atropine at concentrations between 0.01 µM to 10 µM were tested for their spasmolytic effects on the spontaneous contraction of rabbit ileum.

Effect of citral, the extracts of C. citratus (LE, SE and RE) and atropine on ACh-induced contraction

Increasing doses of ACh ranging from 10⁻⁷ M to 10⁻² M were added into the bath and the change in the strength of contractions recorded. The dose-response curve for ACh was then established and the optimal dose of 10⁻³ M was selected to be used for the subsequent experiments. Upon the establishment of a stable spontaneous contraction, the optimal dose of ACh (10⁻³ M) was added and the resulting contraction was recorded. The preparation was then washed

several times, and when the spontaneous contraction was re-established, different doses of either citral at concentrations between 0.061 mM to 15.6 mM, the extracts ranging from 0.001 mg/mL to 1 mg/mL or atropine (positive control) at 0.01 μ M to 1 μ M, respectively, were added into the bath, 5 minutes prior to the addition of ACh (10^{-3} M). The response obtained in the presence of the test materials was expressed as a percentage of inhibition of the maximal force of contraction induced by the optimal dose of ACh. The resulting concentration-response curve was compared to the control curve obtained without the test materials.

Effect of citral, the extracts (LE, SE and RE) and verapamil on KCl-induced contraction

For the determination of calcium channel blocking activity of the test substances, high K^+ (80 mM) was used to induce a maximum sustained contraction as described by Ghayur and Gilani (2005) and Gilani *et al.* (2005c). Test materials were then added in a cumulative fashion to obtain the concentration-dependent inhibitory responses. Verapamil (0.01 μ M to 3 μ M), a potent calcium channel blocker was used as a positive control. The responses obtained were expressed as a percentage of inhibition of the KCl-induced contraction and the concentration-response curves were then plotted.

Characterisation of the spasmolytic action of the test materials

Test materials that showed prominent consistent spasmolytic effects were further investigated for their modes of action that include the possible influence of nitric oxide and calcium antagonistic activities. These experiments were performed only on selected concentrations of citral at 0.488 mM, 0.976 mM, 1.952 mM, 3.9 mM, 7.8 mM and LE at 0.01 mg/mL, 0.03 mg/mL, 0.1 mg/mL, 0.3 mg/mL and 1 mg/mL as both of these test materials showed more consistent spasmolytic effects on the spontaneous, as well as ACh- and KCl-induced contractions.

Nitric oxide pathway

In order to disrupt the NO pathway, L-NAME at 1 μ M (Estrada-Soto *et al.*, 2007) was added into the tissue bath, incubated for 30 minutes and the ileum was allowed to contract spontaneously. Test materials were then added cumulatively into the organ bath and the change in the spontaneous contraction was observed. Dose-response curves of the percentage inhibition of the contraction induced by the test materials in the presence of L-NAME were then plotted and compared with the concentration-response curve obtained in the absence of L-NAME.

Calcium antagonistic activity

To confirm the Ca^{2+} blocking activity of the test substances, the tissues were allowed to first stabilise in normal Tyrode's solution, which was subsequently replaced with Ca^{2+} -free Tyrode's solution containing EDTA (0.1 mM). The ileal strips were kept in this solution for 30 minutes. This method was adopted from Estrada-Soto (2007) with slight modifications. EDTA was used so as to remove any calcium that might have been released from the tissues. The solution was then replaced with K^+ -rich and Ca^{2+} -free Tyrode's solution with the following composition (mM): KCl (50), NaCl (91.04), $MgCl_2$ (1.05), $NaHCO_3$ (11.9), NaH_2PO_4 (0.42), glucose (5.5) and EDTA (0.1). Following an incubation period of 30 minutes, a control concentration-response curve for calcium

was obtained by adding CaCl_2 in increasing doses, in the range of 0.03 mM to 30.0 mM. This CaCl_2 concentration response experiment was repeated but in the presence of the optimal spasmolytic doses of citral (1.952 mM) and LE (1 mg/mL) for 30 minutes prior to the addition of CaCl_2 . Another experiment was performed in the same manner but in the presence of verapamil (1 μM) which acted as a positive control.

The concentrations of drugs such as atropine and verapamil in the present study were selected based on similar works found in the literature (Gilani *et al.*, 2000; Gilani *et al.*, 2005a; Gilani *et al.*, 2005b; Gilani *et al.*, 2006; Estrada-Soto *et al.*, 2007; Gilani *et al.*, 2008)

Statistical Analysis

Relative changes of the contractile activity of the ileum in response to the test materials and/or drugs to the basal level were calculated as percentage, and all values were presented as mean \pm S.E.M. of $n = 4$ to 5 rabbits for each experiment. Statistical significance was estimated by paired Student's *t*-test and a *P* value ≤ 0.05 was regarded to be significant.

Results

Spontaneous contraction

The frequency of the spontaneous contraction of the ileum varied from one ileal strip to another, ranging from 9 to 12 contractions/min. The test materials did not significantly alter the frequency of contraction (Fig. 2). Meanwhile, as shown in Figure 3a, citral at doses ranging from 0.061 mM to 15.6 mM, inhibited the spontaneous contraction of the rabbit ileum, decreasing the contraction from $98.36 \pm 24.05\%$ to $2.94 \pm 26.01\%$ ($P < 0.05$) with IC_{50} value of 0.093 mM. The results obtained showed a similar pattern to that for atropine (Fig. 3c). The extract of leaves (LE) at 0.001 mg/mL to 1 mg/mL demonstrated a slightly weaker spasmolytic effect, reducing the spontaneous contraction by up to $54.1 \pm 4.34\%$ in a dose-dependent manner. The IC_{50} for LE was 1.299 mg/mL. The extract from root did not show significant reduction in the contraction at all the doses used. The extract from stems (SE) significantly reduced the contraction at the highest dose used, but the overall effects were not consistent (Fig. 3b).

ACh-induced contraction

Citral was found to significantly inhibit the submaximal contraction induced by ACh (10^{-3} M) from 23.1 ± 7.27 to $79.61 \pm 9.6\%$ (Fig. 4a) with IC_{50} value of 0.29 mM. The leaf extract caused smaller inhibitions of the ACh-induced contraction which increased gradually to about 40%, with a significant reduction observed at the highest dose used. Interestingly, SE caused a biphasic response with spasmolytic response being greater at the lower and highest doses. At the highest dose of 1 mg/mL, SE with IC_{50} value of 1 mg/mL caused a greater reduction in contraction than that showed by LE (50% versus 40%). Meanwhile, RE did not cause significant spasmolytic effect at all the doses used. As expected, atropine ($\text{IC}_{50} = 0.149 \mu\text{M}$) suppressed the ACh-induced contraction the most, inhibiting it by up to 97% (Fig. 4b).

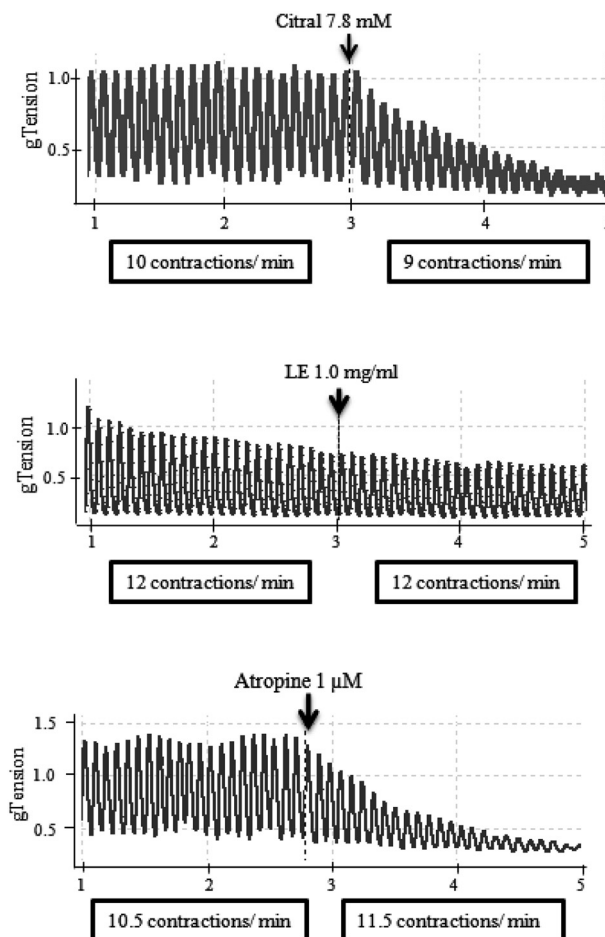


Fig. 2. Representative tracings showing the effects of a selected concentration of citral, leaf extracts (LE) and atropine on the frequency as well as the strength of contraction of spontaneously contracting isolated rabbit ileum.

KCl-induced contraction

Figure 5a shows that citral significantly reduced the contraction induced by KCl. The IC_{50} for citral in this experiment was found to be 0.163 mM. The percent inhibition was increased from $2.27 \pm 0.15\%$ to $79.55 \pm 0.69\%$ at the doses tested. Similarly, the LE also inhibited the KCl-induced contraction in a dose-dependent manner (Fig. 5b). The sustained contraction induced by KCl was attenuated from $1.49 \pm 0.12\%$ to $43.88 \pm 0.26\%$ with significant effects observed at concentrations of 0.1, 0.3 and 1 mg/mL, respectively. Weaker and non-significant inhibitory effects (<20%) were observed with SE and RE. As expected, verapamil significantly reduced the contractions induced by KCl by up to 90% at the highest concentration used with IC_{50} of 0.295 μ M (Fig. 5c).

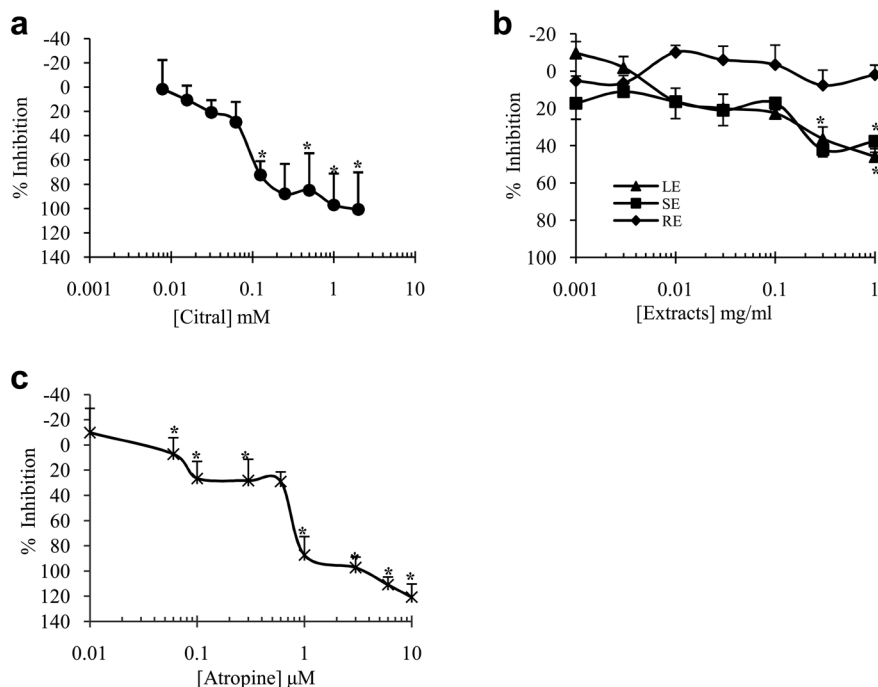


Fig. 3. Effect of (a) citral (b) extracts from leaves (LE), stems (SE) and roots (RE) of *C. citratus*, and (c) atropine on the spontaneous contraction of rabbit ileum. Values are shown as means \pm S.E.M. with $n = 6$ strips of ileum from 4 rabbits, *, $P < 0.05$ compared with vehicle control.

Characterisation of the spasmolytic action of the test materials

Nitric oxide pathway

The dose-dependent inhibition of the spontaneous contraction caused by citral at concentrations of 0.488 mM, 0.976 mM and 1.952 mM were found to be significantly (*, $P < 0.05$) decreased by L-NAME. However, above the concentrations of 1.952 mM of citral, L-NAME did not significantly attenuate the effects of citral, with the inhibitory effect of citral almost reaching the level observed without L-NAME (Fig. 6a). In contrast, L-NAME did not alter the relaxant effect of the extract from leaves of *C. citratus* at all concentrations used (Fig. 6b).

Calcium antagonist test

When the ileal strips were incubated in K^+ -rich- Ca^{2+} -free Tyrode's solution they showed very weak contractile activity. However, addition of increasing doses of $CaCl_2$ restored their contractile activity in a dose-dependent manner. Pre-treating the ileum with citral almost abolished the dose-response curve for Ca^{2+} (Fig. 7a). An unexpectedly greater tone was initially observed after pre-treating the ileum with citral. The ileal strips used for citral and without citral were different and there may have been some variations in the basal tone of the strips thus, resulting in this unexpected observation. The leaf extract reduced the effect of $CaCl_2$ addition, and caused a rightward shift of the Ca^{2+} dose-response curve (Fig. 7b) suggesting a similar albeit a lesser effect

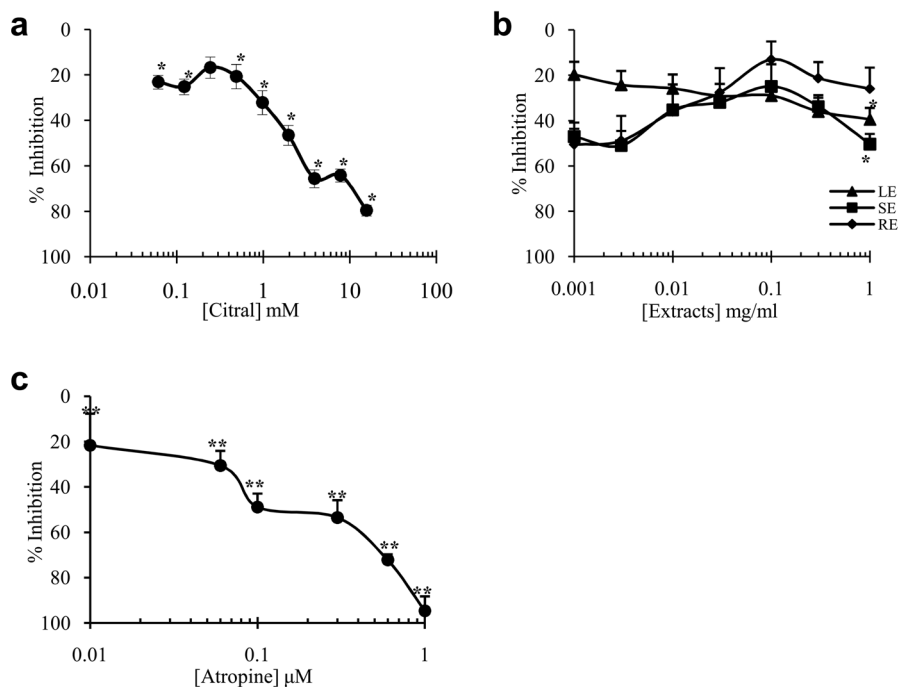


Fig. 4. The % inhibition of ACh-induced contraction of rabbit ileum caused by (a) citral, (b) extracts from leaves (LE), stems (SE) and roots (RE) of *C. citratus*, and (c) atropine. Values are shown as means \pm S.E.M. with $n = 6$ ileal strips from 5 rabbits. *, $P < 0.05$; **, $P < 0.001$ compared to ACh-induced tone.

compared to citral. Verapamil, acting as the positive control, showed a greater effect than citral and completely inhibited the contraction induced by CaCl_2 (Fig. 7c).

Discussion

The present study showed that citral and LE significantly inhibited the spontaneous contraction of the visceral smooth muscle preparations in a dose-dependent manner. However, since the preparation used were whole ileal strips, it was not possible to ascertain whether the test substances inhibited the longitudinal muscle, circular muscle or both. In contrast, the RE did not show significant inhibition on the spontaneous contraction. As for the SE, although it caused a significant inhibition at the highest dose used, it did not produce a consistent dose-response pattern. It has been well established that the spontaneous contraction of the intestinal smooth muscle is due to cycles of depolarisation involving a fast influx of Ca^{2+} which increases the cytosolic Ca^{2+} and subsequently activates the contractile elements. The increase in the cytosolic Ca^{2+} can occur either via influx of the ion through the voltage-operated calcium channel (VOCC) or release of calcium from sarcoplasmic reticulum (McDonald *et al.*, 1994; Elorriaga *et al.*, 1996; Bolton *et al.*, 1999; Dar and Channa, 1999). The frequency of the contractions was not altered by any of the test substances suggesting that they do not modify the frequency of the spontaneous

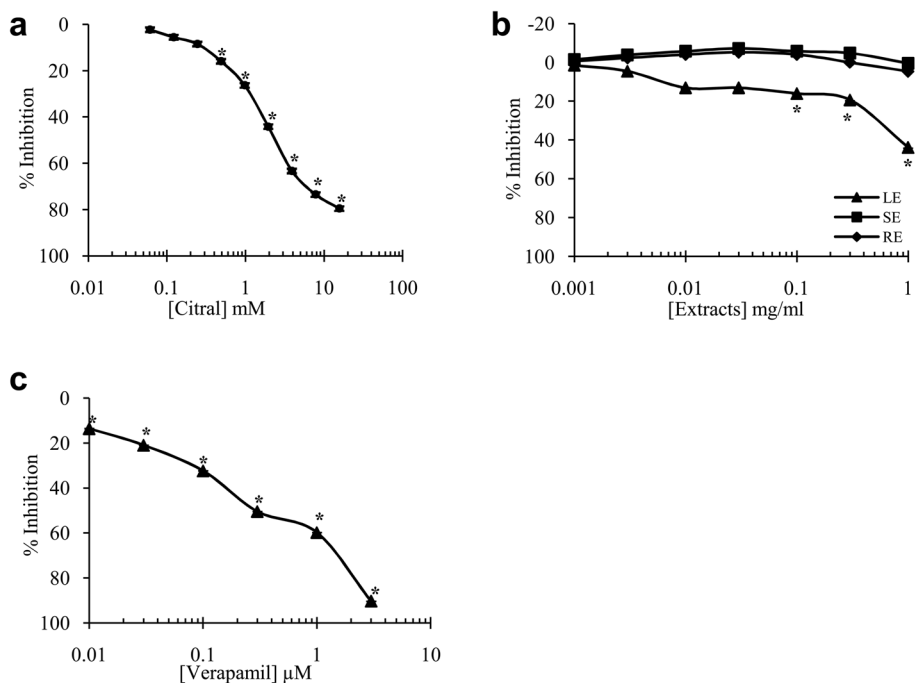


Fig. 5. The % inhibition of KCl-induced contraction of rabbit ileum by (a) citral, (b) extracts from leaves (LE), stems (SE) and roots (RE) of *C. citratus* and (c) verapamil. Values are shown as means \pm S.E.M. with $n = 6$ ileal strips from 5 rabbits. *, $P < 0.05$ compared to KCl-induced tone.

depolarisation from the pacemaker cells.

Furthermore, this study showed that citral and LE can also reduce the contractile effect of ACh on the visceral smooth muscle. These effects were similar, albeit smaller, to that of atropine, the muscarinic receptor blocker, suggesting that citral and LE may be acting as an antagonist to the muscarinic receptor. Activation of the muscarinic receptor induces activation of a G protein-coupled receptor which activates phospholipase-C to produce inositol-1,4,5, triphosphate (IP3) and diacylglycerol (DAG). These molecules mediate Ca^{2+} release or entry into smooth muscle cells (Macara and Rico, 1992; Bolton *et al.*, 1999; Kirschstein *et al.*, 2009). Thus, citral and LE may block the production of IP3 and/ or block the release of Ca^{2+} from the storage sites. Further studies using appropriate blockers against these second messengers could better elucidate their involvement.

Potassium, at doses greater than 30 mM, is known to cause smooth muscle contraction through opening of voltage-operated L-type calcium channels, allowing influx of extracellular Ca^{2+} . The high potassium concentration causes depolarisation of the tissue and produces a sustained contraction which would enable a dose-dependent inhibitory response data to be obtained. Substances that inhibit the K^{+} -induced contraction are considered as blockers of Ca^{2+} influx (Brankovic *et al.*, 2009; Kirschstein *et al.*, 2009).

In the present study, the sustained contraction of the muscle caused by high concentration of

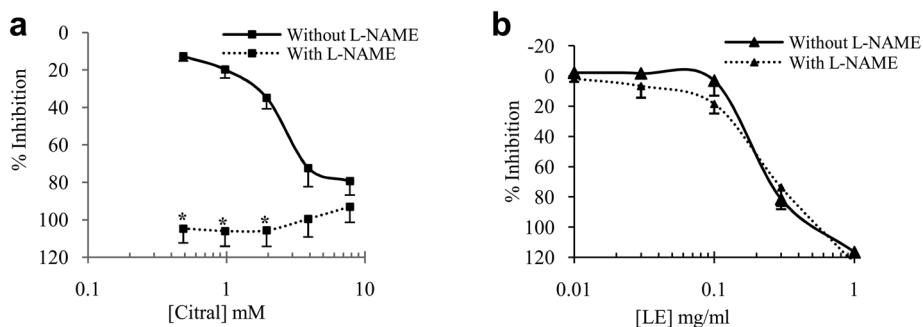


Fig. 6. The effect of (a) citral and (b) extract from leaf (LE) of *C. citratus* on the spontaneous contraction of ileum smooth muscle in the presence and absence of L-NAME. Values are shown as means \pm S.E.M. with $n = 6$ ileal strips from 5 rabbits. **, $P < 0.05$ compared with the percentage of contraction in the absence of L-NAME.

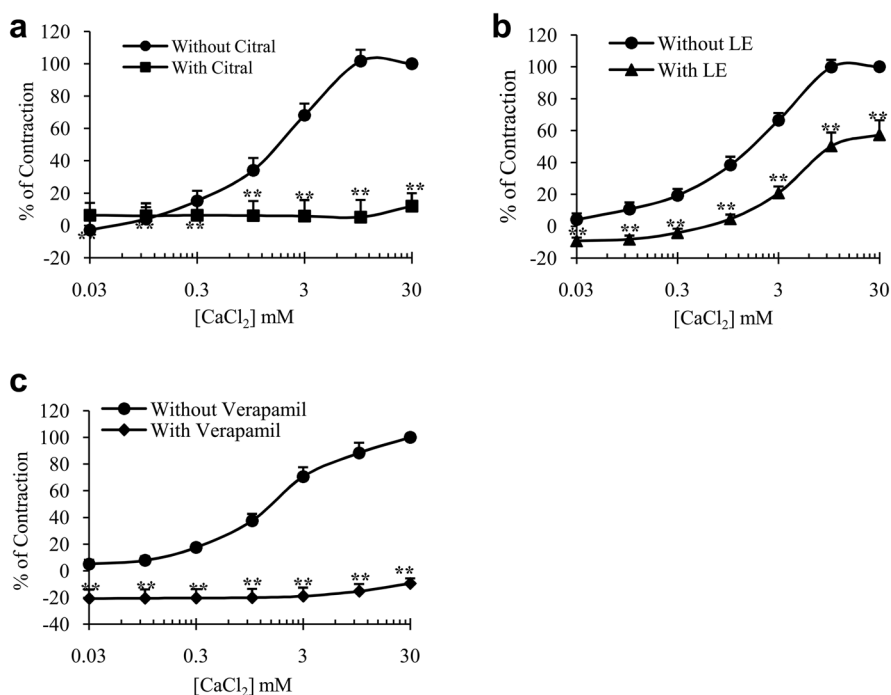


Fig. 7. Dose-response curves for calcium in the absence and presence of (a) citral, (b) extract from leaf (LE) and (c) verapamil on the KCl-induced contraction of smooth muscle of ileum in K^+ -rich- Ca^{2+} -free Tyrode's solution. Values are shown as means \pm S.E.M. with $n = 6$ ileal strips from 5 rabbits. **, $P < 0.001$ compared with the contraction induced by KCl in increasing doses of calcium without test materials.

K^+ was attenuated by citral and LE in a concentration-dependent manner. These results were similar to what was obtained with verapamil, a standard VOCC antagonist. The inhibitory effect of citral was highly comparable to verapamil; with 80% inhibition by citral compared to 90% by verapamil at the highest concentration used. Meanwhile, the LE showed only a maximum

inhibition of 44%. The present findings demonstrated that the inhibitory effects of citral and LE on the visceral smooth muscle were highest on the spontaneous contraction followed by the KCl-induced contraction and then the ACh-induced contraction.

The inhibition of the K⁺-induced contraction of rabbit ileum preparations by citral and LE may reflect the restriction of Ca²⁺ entry via VOCC. This postulation was further supported by our findings that when the tissue was pre-treated with citral or LE in K⁺ rich-Ca²⁺-free Tyrode's solution, a rightward shift in the concentration response curve of CaCl₂ was observed. A similar but greater effect was produced by verapamil. Citral at 1.952 mM concentration almost abolished the contraction of smooth muscle elicited by addition of Ca²⁺ that was comparable with verapamil at 1 μM. Extract of leaves at a concentration of 1 mg/mL also reduced, but to a lesser extent, the effect of Ca²⁺ on the contraction of smooth muscle of rabbit ileum in K⁺ rich-Ca²⁺-free Tyrode's solution. The findings from these two studies strongly suggest the presence of Ca²⁺ antagonistic constituent(s) in the test substances.

The second messenger, nitric oxide, is an important candidate for mediating non-adrenergic and non-cholinergic smooth muscle relaxation in the gastrointestinal tract (Estrada *et al.*, 1999; Takahashi, 2003; Estrada-Soto *et al.*, 2010). Therefore, we explored the possibility that NO was involved in the antispasmodic activity displayed by citral and LE on the spontaneous contractions. Pre-treating the ileal strips with L-NAME significantly lowered the inhibitory effect of lower doses of citral, suggesting that NO may be responsible for its relaxant effects. However, as the concentration of citral was increased to more than 1.952 mM the effect of L-NAME decreased. Since only one dose of L-NAME (1 μM) was used, it was possible that at higher concentrations citral was able to overcome the effect of L-NAME. Thus, the % inhibition on the muscle contraction became similar to the condition without L-NAME. Meanwhile, the spasmolytic effect of the LE was not attenuated by the presence of L-NAME at all. These findings indicate that NO may be mediating the relaxant effect induced by citral but not that induced by LE. Thus, it appears that LE may contain other active chemical components other than citral which warrant further studies.

Conclusion

The present study demonstrated for the first time, that citral is able to produce spasmolytic activity in isolated rabbit ileum. Citral appeared to induce relaxation through NO pathway and blockade of calcium channels via VOCC and/or receptor-operated calcium channel (Cayne *et al.*, 2005). Furthermore, citral may also act by directly blocking the muscarinic receptor and possibly by inhibiting the production and/or action of the second messenger, IP₃ causing the visceral smooth muscle to relax. The leaf extract also exhibited spasmolytic effect which may act via blockade of calcium channels and muscarinic receptor but not via the NO pathway. The findings from this study provided support for the use of this plant in traditional medicine and contributed important scientific evidence that merit further investigations. The findings also provided evidence that while citral may be the major active constituent of *C. citratus*, there may also be other active chemical constituents present in the plant which can cause inhibition of visceral smooth muscle contraction. Further studies are also required to ascertain the specific layer of

muscle that is affected by the test materials and whether L-NAME interferes with the action of the test substances on the ACh- and the K⁺-induced contractions.

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