Title:

Inhibition of compound action potentials in the frog sciatic nerve by inchinkoto, a traditional Japanese medicine used for oral mucositis

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Abbreviations:

4'-HA, 4'-hydroxyacetophenone

ANOVA, analysis of variance

CAP, compound action potential

CGA, chlorogenic acid

DMSO, dimethyl sulfoxide

DRG, dorsal root ganglion

IC_{50}, half-maximal inhibitory concentration

n_H, Hill coefficient

R^2, the square of correlation coefficient

SEMs, standard errors of means

Abstract

***Objective*:** This study aimed to determine the effects of traditional Japanese (Kampo) medicines used to treat oral mucositis on nerve conduction.

***Methods*:** The effects of Kampo medicines, crude drugs, and chemical compounds on compound action potentials (CAPs) were analyzed using extracellular recordings in frog sciatic nerves.

***Results*:** Among the Kampo medicines, inchinkoto demonstrated the most significant reduction in CAP amplitude, with a half-maximal inhibitory concentration (IC_{50}) of 5.4 mg/mL. Hangeshashinto, shosaikoto, hochuekkito, and juzentaihoto also showed a significant reduction. Regarding inchinkoto, Artemisiae Capillari Spica (artemisia) was the most effective crude drug, with an IC_{50} of 4.2 mg/mL for CAP amplitude reduction, whereas Gardeniae Fructus (gardenia) exerted no significant effect. However, the combined use of artemisia and gardenia reduced the CAP amplitude more effectively than artemisia alone, indicating a synergistic interaction. The chemical ingredient eugenol from artemisia administered at 1 and 3 mmol/L reduced CAP amplitude, whereas other chemical ingredients administered at 0.1 and 1 mmol/L had no significant effects.

***Conclusions*:** Inchinkoto exhibited the most effective reduction in CAP amplitude in the sciatic nerve of frogs, primarily through the action of artemisia, with potential synergistic interaction between artemisia and gardenia.

1. Introduction

Oral mucositis arises from diverse factors, including infections, inflammation, allergies, and nutritional issues, and it is prevalent among denture and orthodontic appliance users [1–3]. Patients undergoing chemotherapy and radiation therapy for head and neck cancer are also prone to oral mucositis [4]. The condition induces severe pain, leading to diminished quality of life for affected individuals [5,6]. Traditional Japanese (Kampo) medicines have shown effectiveness in treating oral diseases [7,8]. Notably, byakkokaninjinto, hangeshashinto, hochuekkito, inchinkoto, juzentaihoto, orengedokuto, orento, and shosaikoto are used to treat oral mucositis in Japan [9,10]. Among these medicines, hangeshashinto has been reported to exhibit analgesic effects in an oral ulcer rat model [11]. Processed ginger (steamed rhizome of *Zingiber officinale* Roscoe, Zingiberaceae) and glycyrrhiza (root or stolon of *Glycyrrhiza uralensis* Fisher, Leguminosae) are the crude drugs incorporated into hangeshashinto [11]. Chemical components of processed ginger ([6]-gingerol and [6]-shogaol) and glycyrrhiza (isoliquiritigenin) have been reported to inhibit voltage-gated Na^+ channels that play a pivotal role in the analgesic effects of hangeshashinto [12,13]. Although hangeshashinto's analgesic effects have been explored, the analgesic potential and mechanisms of other Kampo medicines used for oral mucositis remain insufficiently investigated.

Numerous plant-derived chemicals have been identified for their ability to inhibit fast-conducting and voltage-gated Na^+ channel blocker tetrodotoxin (TTX)-sensitive compound action potentials (CAPs) in the frog sciatic nerve, exemplified by capsaicin in hot peppers [14] and menthol in peppermint [15]. Given that Kampo medicines consist of various plant-derived crude drugs, they likely possess the capability to inhibit nerve action potential conduction. Matsushita et al. [16] reported that daikenchuto, rikkosan, kikyoto, rikkunshito, shakuyakukanzoto, and kakkonto, used to alleviate various pain types,

inhibited CAP amplitudes. Considering the analogous action of local anesthetics in pain alleviation through conduction inhibition [17–19], the present study aimed to determine the effects of Kampo medicines used for oral mucositis on CAP peak amplitudes recorded from the frog sciatic nerve using extracellular recording.

2. Materials and methods

2.1. Animals

Black-spotted pond frogs (*Pelophylax nigromaculatus*) were provided by the Amphibian Research Center, Hiroshima University (Hiroshima, Japan) and Hamamatsu Seibutsu Kyozai (Shizuoka, Japan). The Animal Care Committee of Fukuoka Dental College approved this study (certification No. 20009), which was conducted according to the Guiding Principles for the Care and Use of Animals in the Field of Physiological Science of the Physiological Society of Japan. All efforts were made to minimize animal suffering and the number of animals used.

2.2. Preparation of frog sciatic nerves

A previously established method was used to obtain frog sciatic nerve preparations [16,20,21]. Briefly, under 2% isoflurane anesthesia (Viatris, Canonsburg, PA, USA), frogs (both sexes) were decapitated and pithed. The sciatic nerve, dissected from the lumbar plexus to the knee in modified Ringer solution, was carefully desheathed under a binocular microscope. Subsequently, the nerve was placed on five platinum wires affixed to a transparent acrylic plate, with both ends secured to the wires with threads. The sciatic nerve was immersed in a beaker containing modified Ringer solution (pH 7.0) comprising (mmol/L) NaCl (115.5), KCl (2.0), $CaCl_2$ (1.8), Na_2HPO_4 (1.3), and NaH_2PO_4 (0.7).

2.3. Recordings of CAPs from sciatic nerve fibers

As previously described [16,20,21], the acrylic plate with the sciatic nerve was transferred from modified Ringer solution to an empty beaker. CAPs were recorded in air using a preamplifier (Model LI-75A, NF Electronics Instruments, Yokohama, Japan). Two platinum wires stimulated the nerve at 1 Hz through a stimulator, with rectangular pulses lasting 0.1 ms and varying in strength. Two other wires recorded CAPs. To prevent the sciatic nerve from drying out, this process was rapidly performed within 30 s. As a control, CAP recordings were performed in Ringer solution over the first 10 min, with a 2 min interval between recordings. For assessing the impact of drugs on CAPs, the nerve was exposed to drug-containing Ringer solution for the subsequent 20 min, with CAPs recorded every 2 min. Following drug treatment, the sciatic nerve was returned to drug-free Ringer solution, and CAPs were recorded every 2 min for 10 min in total, representing the washout period. CAPs were monitored on a storage oscilloscope and simultaneously recorded on a thermal array recorder. CAP waveform data were saved using a data logger (midi LOGGER GL900, GRAPHTEC, Yokohama, Japan). Electrical stimulation of the sciatic nerve generated a CAP following a stimulus artifact.

CAP peak amplitudes were measured as the difference between baseline and peak CAP levels, as previously described [16,21]. The peak amplitude of the maximal CAP was analyzed, considering that peak amplitude increases with the strength of electrical stimulation. The average CAP peak amplitudes recorded for 10 min before drug treatment served as the control. The relative CAP amplitude (%) was calculated as the ratio of each CAP amplitude to the control. Conduction velocity values were determined using the fifth platinum wire on the acrylic plate as an additional electrical stimulation site, measuring the time change between the stimulus artifact and the CAP peak. All experiments were

conducted at room temperature.

2.4. Drugs

Byakkokaninjinto (Lot No. 2190034010), hangeshashinto (Lot No. 2180014010), hochuekkito (Lot No. 2190041010), inchinkoto (Lot No. 2190135010), juzentaihoto (Lot No. 2190048010), orengedokuto (Lot No. 2170015010), orento (Lot No. 2190120010), and shosaikoto (Lot No. 2200009010), all obtained from Tsumura & Co. (Tokyo, Japan), were prepared as a spray-dried powder from a hot-water extract without excipients. Inchinkoto composed three crude drugs: Artemisiae Capillari Spica (artemisia; the spike of *Artemisia capillaris* Thunberg: 50.0%), Gardeniae Fructus (gardenia; the fruit of *Gardenia jasminoides* Ellis: 37.5%), and Rhei Rhizoma (rhubarb; the rhizome of *Rheum palmatum* Linné: 12.5%). Spray-dried extract powders of artemisia (Lot No. 2191085010), gardenia (Lot No. 2211044010), and rhubarb (Lot No. 2211028010), provided by Tsumura & Co., were also prepared.

The powdered extracts of Kampo medicines and crude drugs were suspended in Ringer solution at concentrations of 0.3–10 mg/mL and stirred gently for 1 h in the dark. The suspension underwent filtration through qualitative filter paper (grade No. 1; nominal rating: 6 μm; ADVANTEC, Tokyo, Japan), with separation of insolubles was and use of the supernatant. Weighing the filter papers before and after filtration indicated the loss of powder on the filter paper in Ringer solution containing Kampo medicines and crude drugs (Tables 1 and 2).

The chemical compounds known to be present in artemisia, namely caffeic acid, chlorogenic acid (CGA), esculetin, eugenol, 4'-hydroxyacetophenone (4'-HA), scopoletin (all obtained from Tokyo Chemical Industry Co., Tokyo, Japan), and scoparone (Sigma-Aldrich, St. Louis, MO, USA) [22], were initially dissolved in dimethyl sulfoxide (DMSO; FUJIFILM Wako Pure Chemical Corporation Osaka,

Japan), followed by dilution to the final concentration in Ringer solution prior to use, with the final DMSO concentration kept below 1%, as 1% DMSO does not affect CAPs [14,15]. The pH of the solution containing the drugs was adjusted to 7.0 using NaOH.

2.5. Statistical analysis

Data are presented as means ± standard errors of means (SEMs). All statistical analyses were conducted using JASP [23], employing paired Student's *t*-tests and one-way analysis of variance (ANOVA) with post-hoc Tukey–Kramer tests. Sigmoidal curves illustrating the concentration–response relationship between relative CAP amplitude and drug concentration were constructed according to the Hill equation (for inchinkoto and artemisia), and regression lines (for hangeshashinto and eugenol) were determined using DeltaGraph 6.0 (Red Rock Software, Salt Lake City, UT, USA). Statistical significance was set at $p < 0.05$. In all cases, n denotes the number of sciatic nerves examined.

3. Results

The effects of Kampo medicines, crude drugs, and chemical compounds contained in the Kampo medicine inchinkoto on CAPs were assessed in 192 sciatic nerves from 28 frogs, with an average CAP amplitude of 17.8 ± 0.7 mV. In some sciatic nerves, CAPs exhibited average conduction velocity values of 31.0 ± 2.5 m/s (n = 44). These values are comparable to those reported previously [14–16].

3.1. Effects of Kampo medicines used for oral mucositis on frog sciatic nerve CAPs

Eight Kampo medicines (byakkokaninjinto, hangeshashinto, hochuekkito, inchinkoto, juzentaihoto, orengedokuto, orento, and shosaikoto) used for treating oral mucositis were examined for their impact

on frog sciatic nerve CAP amplitudes. Figure 1A illustrates CAP recordings before treatment, after 20 min exposures to 5 mg/mL Kampo medicines, and in drug-free Ringer solution at 8–10 min post-treatment. Figure 1B depicts the average time courses of changes in CAP peak amplitude following exposures to the eight Kampo medicines containing Ringer solutions relative to the control. The Kampo medicines inchinkoto, hangeshashinto, shosaikoto, hochuekkito, and juzentaihoto reduced CAP amplitudes almost maximally after 20 min of treatment. Figure 1C summarizes the effects on CAP amplitudes, with inchinkoto identified as the most effective medicine in reducing these amplitudes, although with no significant difference compared with hangeshashinto, shosaikoto, hochuekkito, and juzentaihoto. Orento, byakkokaninjinto, and orengedokuto did not significantly reduce CAP amplitudes.

Given inchinkoto's notable inhibitory effect on CAP amplitudes, its concentration dependency was examined. Exposure to 10 mg/mL inchinkoto for 20 min resulted in a larger reduction in CAP amplitude compared with 1 mg/mL inchinkoto (Fig. 2A). Figure 2B shows the average time courses of changes in CAP peak amplitudes across a 0.3–10 mg/mL inchinkoto concentration range. Inchinkoto-induced reduction was almost maximal following 20 min exposures and was concentration-dependent, with a concentration–response curve (Fig. 2C) revealing an estimated half-maximal inhibitory concentration (IC_{50}) value of 5.4 mg/mL. Hangeshashinto (5 mg/mL) also reduced CAP amplitude, and its concentration dependency at 1, 5, and 10 mg/mL tended toward increased inhibition with higher concentrations, although there was no significant difference between 5 and 10 mg/mL hangeshashinto (Fig. 2D). Although shosaikoto reduced CAP amplitude in a comparable manner to that of hangeshashinto, its concentration dependency was not examined in this study.

3.2. Effects of crude drugs in inchinkoto on CAP amplitudes

Inchinkoto comprises three crude drugs: artemisia, gardenia, and rhubarb [9,24]. Examination of artemisia revealed that 10 mg/mL artemisia caused a larger, almost reversible, reduction in CAP amplitude compared with 5 mg/mL artemisia, with only slight effects observed at 1 mg/mL (Fig. 3A). Figure 3B shows average time courses of CAP amplitudes following exposure to artemisia (0.3–10 mg/mL) relative to the control, with these amplitudes being reduced in a concentration-dependent manner by artemisia and attaining almost maximal levels following 20 min of exposure. Figure 3C shows the concentration–response curve of artemisia (0.3–10 mg/mL) with reduced CAP amplitude, where the estimated IC_{50} of artemisia was 4.2 mg/mL. Rhubarb and gardenia (each 5 mg/mL), ingredients of inchinkoto, were also investigated, with rhubarb partially reducing CAP amplitude and gardenia causing only a slight reduction (Fig. 4A). Figure 4B displays the reduction in CAP amplitude due to these three crude drugs relative to the control, with almost maximal reduction reached after 20 min of treatment. Comparing CAP amplitudes following 5 mg/mL crude drug exposures for 20 min with control values, rhubarb significantly reduced CAP amplitude, whereas gardenia did not (Fig. 4C). Among the crude drugs, artemisia exhibited the most potent CAP amplitude reduction effect.

Crude drugs contain multiple bioactive chemical compounds, which potentially synergize to exert a more pronounced facilitatory effect compared with each component alone [25]. To explore potential interactions among crude drugs in inchinkoto, mixture pairs of artemisia, gardenia, and rhubarb were examined. Given the estimated quantities of artemisia (5 mg/mL), rhubarb (1 mg/mL), and gardenia (4 mg/mL) in a 10 mg/mL inchinkoto solution, we prepared three mixtures of crude drugs: 5 mg/mL artemisia + 4 mg/mL gardenia, 5 mg/mL artemisia + 1 mg/mL rhubarb, and 4 mg/mL gardenia + 1 mg/mL rhubarb. Artemisia-containing mixtures reduced CAP amplitude almost reversibly, whereas a mixture without artemisia produced only slight reductions (Fig. 5A). Figure 5B illustrates the average

time course of CAP amplitudes following 20 min mixture exposures relative to the control, with CAP amplitude reduction being almost maximal at 20 min for all treatments. Figure 5C summarizes the average CAP amplitudes following 20 min exposures relative to the control. Among the artemisia-containing solutions, all of which had the same artemisia concentration, the artemisia + gardenia mixture reduced CAP amplitudes more than artemisia alone, with the reduction being comparable to that caused by inchinkoto. However, no significant difference was observed between CAP amplitude reductions exerted by artemisia + gardenia and artemisia + rhubarb.

3.3. Effects of chemical compounds in artemisia on CAP amplitudes

Artemisia contains over 70 chemical compounds, including coumarins, flavonoids, chromones, organic acids, and alkaloids [22,26,27]. Notably, the coumarin compounds scoparone and scopoletin, with relatively high contents in artemisia (0.035–14.800 and 0.031–0.054 mg/g, respectively) [22], were reported to contribute to the hepato-protective and anti-inflammatory effects of inchinkoto [28]. In this study, we examined their impact on CAP amplitude. Additionally, the coumarin compound esculetin, which is structurally related to scoparone and scopoletin (Fig. 6A), was also examined, despite its low content (trace amount to 0.013–0.021 mg/g) [22]. Furthermore, the organic acids CGA and caffeic acid as well as 4'-HA were examined for their reducing effects on CAP amplitude, given their relatively high content in artemisia (0.520–27.972, 0.101–6.495, and 0.300–8.795 mg/g, respectively). Moreover, the vanilloid component eugenol (0.068 mg/g in artemisia [22]), known for inhibiting CAP amplitude [14], was assessed. The chemical structures of the examined compounds are depicted in Fig. 6A.

Upon exposure of the sciatic nerve to seven chemical compound types (each at 1 mmol/L) for 20 min, CAP amplitudes were either unaltered or slightly reduced, except for eugenol, which caused a

reversible reduction (Fig. 6B). Figure 6C summarizes the average CAP peak amplitudes following exposure to chemical compounds at 0.1 and 1 mmol/L (eugenol was tested at 0.1–3 mmol/L) for 20 min relative to the control. Except for eugenol, no chemical compound significantly reduced CAP amplitudes, whereas eugenol treatment at 1 and 3 mmol/L resulted in a significant reduction. The IC_{50} value, estimated via linear regression, was 1.2 mmol/L ($R^2 = 0.953$), consistent with previous report [14].

4. Discussion

In this study, inchinkoto exposure resulted in the most substantial reduction in frog sciatic nerve CAP amplitude among the tested Kampo medicines, although no significant difference was observed compared to hangeshashinto, shosaikoto, hochuekkito, and juzentaihoto. Test solutions were prepared by suspending Kampo medicines in Ringer solution at 5 mg/mL, followed by filtration and use of the supernatant. Despite the final concentrations of these medicines being < 5 mg/mL, inchinkoto demonstrated superior effectiveness. Although topical application of hangeshashinto (100 mg/mL) reportedly alleviates oral ulcerative mucositis–induced pain [11], the reduction in CAP amplitude caused by hangeshashinto in the present study did not surpass that of inchinkoto, possibly due to differences in concentrations. Orento, byakkokaninjinto, and orengedokuto did not significantly reduce CAP amplitude; filtration during the preparation of drug-containing solutions may have contributed to the lack of reduction, especially for orento and byakkokaninjinto.

Among the crude drugs in inchinkoto, artemisia and rhubarb effectively reduced CAP amplitude, whereas gardenia caused no such reduction. Given that artemisia constitutes approximately 50% of the total ingredients and resulted in the largest reduction in CAP amplitudes among the crude drugs, it may primarily contribute to the inchinkoto-induced reduction. Artemisia and gardenia are known for their

anti-inflammatory, antipyretic, and choleric effects, whereas rhubarb is associated with laxative and blood fluidity improvement effects [9], and gardenia shows antinociceptive effects [29,30]. Artemisia may contribute to analgesic effect production by inhibiting nerve conduction. Kampo medicines, composed of various crude drugs, can induce synergistic effects compared with single crude drugs. Examination of the interactions among artemisia, gardenia, and rhubarb revealed that the artemisia + gardenia mixture treatment led to a larger reduction in CAP amplitude than artemisia alone, with the effect comparable to that of inchinkoto. Although gardenia alone did not significantly reduce CAP amplitude, the artemisia + gardenia mixture was more effective than the other two mixtures. Genipin, a major bioactive component in gardenia [27,31], is recognized as a noncytotoxic crosslinking compound. It enhances the functional properties of proteins by altering their structures through noncovalent interactions, including hydrogen bonds, hydrophobic interactions, and van der Waals forces [32]. Consequently, this conformational stability induced in proteins (e.g., channels or receptors) in the sciatic nerve or chemical compounds contained in the crude drug may contribute to a synergistic effect between artemisia and gardenia in reducing CAP amplitude. Further investigation is necessary to understand this synergistic interaction.

Artemisia contains various chemical compounds, including coumarins, flavonoids, chromones, organic acids, and alkaloids [22,26,27]. The coumarin components scoparone, scopoletin, and esculetin, which are structurally related via their number of methyl groups, did not reduce CAP amplitude following 0.1 and 1 mmol/L treatments. Given that coumarins are a major component of artemisia and are involved in inchinkoto's principal efficacy, specifically its hepatoprotective effect, their effects have been well examined [22,26–28,33]. Coumarins in artemisia were expected to affect nerve conduction. However, coumarins may not play a major role in nerve conduction. CGA and its metabolite caffeic acid

exert antioxidant, antihypertensive, and antinociceptive effects [34,35]; however, they did not reduce CAP amplitudes at 0.1 and 1 mmol/L treatment levels. Although 4'-HA is known for anti-inflammatory and antinociceptive activities [36,37], it similarly did not reduce CAP amplitudes at these levels. We recorded voltage-gated TTX-sensitive and fast-conducting CAPs [14–16,18,38], but the tested chemical compounds may not affect voltage-gated TTX-sensitive Na^+ channels involved in CAP production in frogs. Eugenol, known to reduce frog sciatic nerve CAP amplitude with an IC_{50} value of 0.81 mmol/L [14], also reduced CAP amplitude in the present study, suggesting its involvement, at least partially, in artemisia's reduction of CAP amplitudes, despite its low artemisia content. Identifying the essential chemical component in artemisia for inchinkoto-induced reduction of CAP amplitudes remains a subject for further investigation.

Among the nine distinct voltage-gated Na^+ channels ($Na_v1.1$–$Na_v1.9$) cloned from mammals (e.g., humans, mice, and rats), $Na_v1.1$, $Na_v1.3$, $Na_v1.6$, $Na_v1.7$, $Na_v1.8$, and $Na_v1.9$ are expressed in DRG neurons and may play important roles in nociception [39,40]. In addition, $Na_v1.7$, $Na_v1.8$, and $Na_v1.9$ are implicated in inflammatory and potentially neuropathic pain [40,41]. $Na_v1.1$–$Na_v1.4$, Nav1.6, and $Na_v1.7$ channels are TTX-sensitive, whereas $Na_v1.5$, $Na_v1.8$, and $Na_v1.9$ are TTX-resistant [40,41]. Unlike in mammals, examination of voltage-gated Na^+ channels in amphibians remains limited. Frogs (*Xenopus tropicalis*) are reported to exhibit homologs of $Na_v1.6$ (human SCN8A genes) and $Na_v1.4$ (human SCN4A) but not $Na_v1.8$ (human SCN10A) and $Na_v1.9$ (human SCN11A) [42–44]. In mammals, $Na_v1.6$ is located in the nodes of Ranvier in myelinated neurons in both the peripheral and central nervous systems [40,45] and is also expressed in unmyelinated peripheral axons [46]. Given that $Na_v1.4$ is exclusively expressed in skeletal muscle in humans [41], $Na_v1.6$ may be implicated in the inhibitory effects on CAP amplitudes. Considering the possibility that frogs possess other types of voltage-gated

Na^+ channels distinct from those in mammals, in addition to $Na_v1.6$ [42–44], further investigation is required to determine the specific channels involved in the observed inhibitory effects.

Frog (*Rana pipiens*) sciatic nerves have TTX-sensitive Aα- and Aδ-fibers and TTX-resistant C-fibers [47,48]. Nociceptive information transmission from the periphery to the central nervous system involves action potential conduction in nerve fibers, specifically Aδ- and C-fibers [18]. However, Aδ- and C-fiber CAPs were not able to be isolated from Aα-fiber ones in the frog sciatic nerve, because of markedly smaller CAP peak amplitudes and lower conduction velocity than those of Aα-fibers [47,48]. Therefore, the CAPs recorded in this study were suggested to be voltage-gated TTX-sensitive and fast-conducting (potentially myelinated Aα-fiber–mediated) CAPs [14–16,18,38]. Consequently, we could not compare the effects of the drugs on CAP amplitude among Aα-, Aδ-, and C-fibers. Although this study focused on examining the impact of Kampo medicines used for oral mucositis on nerve conduction, it did not explore the analgesic effects on pain due to oral mucositis. Moreover, the study does not clarify whether Kampo medicines, which were capable of reducing CAP amplitude, alleviated pain associated with oral mucositis. Lidocaine, a commonly used local anesthetic for relieving oral mucosal pain [4,11,12,17], is reported to reduce CAP amplitude with an IC_{50} value of 0.74 mmol/L [49], which is comparable to that of eugenol. Considering these findings, inchinkoto may potentially offer relief from oral mucositis pain.

5. Conclusions

Among the tested Kampo medicines, inchinkoto demonstrated the most effective reduction in CAP amplitudes, with other Kampo medicines, such as hangeshashinto, shosaikoto, hochuekkito, and juzentaihoto, also significantly reducing these amplitudes. Inchinkoto is composed of artemisia,

gardenia, and rhubarb, with artemisia being the primary contributor to inchinkoto-induced CAP amplitude reduction. Moreover, a mixture of artemisia and gardenia was more effective than artemisia alone, whereas gardenia treatment alone did not lead to CAP amplitude reduction. This suggests a potential synergic interaction between artemisia and gardenia. Among the chemical compounds in artemisia, only eugenol treatment reduced CAP amplitudes. Future studies will aim to elucidate crude drug synergic interactions and identify the essential chemical components for CAP amplitude reduction. Such findings may contribute valuable insights for the use of Kampo medicines in alleviating oral mucositis–related pain.

Ethical statement

This study was approved by the Animal Care Committee of Fukuoka Dental College approved this study (certification No. 20009), which was conducted according to the Guiding Principles for the Care and Use of Animals in the Field of Physiological Science of the Physiological Society of Japan.

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CrediT authorship contribution statement

Mayuko Nishimura: Data curation, Formal Analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization; **Suguru Taniguchi:** Data curation, Investigation, Writing – review & editing; **Sachio Tamaoki**: Supervision, Writing – review & editing; **Tsugumi Fujita**: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Supervision,

Visualization, Writing – original draft, Writing – review & editing

Data availability

The data sets generated and analyzed in this study are available from the corresponding author upon reasonable request.

Declaration of competing interest

The authors declare that they have no competing interests.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used Google Translate to check the grammar. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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References

[1] Akintoye SO, Greenberg MS. Recurrent aphthous stomatitis. Dent Clin North Am 2014;58:281–97.

[2] Salerno C, Pascale M, Contaldo M, Esposito V, Busciolano M, Milillo L, et al. Candida-associated denture stomatitis. Med Oral Patol Oral Cir Bucal 2011;16:e139–43.

[3] Peterson MR, Wong PH, Dickson SD, Coop CA. Allergic stomatitis from orthodontic adhesives. Mil Med 2017;182:e1883–5.

[4] Saunders DP, Epstein JB, Elad S, Allemano J, Bossi P, Van De Wetering MD, et al. Systematic review of antimicrobials, mucosal coating agents, anesthetics, and analgesics for the management of oral mucositis in cancer patients. Supportive Care in Cancer 2013;21:3191–207.

[5] Bossi P, Bergamini C, Miceli R, Cova A, Orlandi E, Resteghini C, et al. Salivary cytokine levels and oral mucositis in head and neck cancer patients treated with chemotherapy and radiation therapy. Int J Radiat Oncol Biol Phys 2016;96:959–66.

[6] Al-Ansari S, Zecha JAEM, Barasch A, de Lange J, Rozema FR, Raber-Durlacher JE. Oral mucositis induced by anticancer therapies. Curr Oral Health Rep 2015;2:202–11.

[7] Zheng LW, Hua H, Cheung LK. Traditional Chinese medicine and oral diseases: Today and tomorrow. Oral Dis 2011;17:7–12.

[8] Yamaguchi K. Traditional Japanese herbal medicines for treatment of odontopathy. Front Pharmacol 2015;6:176.

[9] Sunagawa M, Yamaguchi K, Tsukada M, Ebihara N, Ikemoto H, Hisamitsu T. Kampo (traditional Japanese herbal) formulae for treatment of stomatitis and oral mucositis. Medicines 2018;5:130.

[10] Veilleux M-P, Moriyama S, Yoshioka M, Hinode D, Grenier D. A Review of evidence for a therapeutic application of traditional Japanese Kampo medicine for oral diseases/disorders. Medicines 2018;5:35.

[11] Hitomi S, Ono K, Yamaguchi K, Terawaki K, Imai R, Kubota K, et al. The traditional Japanese medicine hangeshashinto alleviates oral ulcer-induced pain in a rat model. Arch Oral Biol 2016;66:30–7.

[12] Hitomi S, Ono K, Terawaki K, Matsumoto C, Mizuno K, Yamaguchi K, et al. [6]-gingerol and [6]-shogaol, active ingredients of the traditional Japanese medicine hangeshashinto, relief oral ulcerative mucositis-induced pain via action on Na^+ channels. Pharmacol Res 2017;117:288–302.

[13] Miyamura Y, Hitomi S, Omiya Y, Ujihara I, Kokabu S, Morimoto Y, et al. Isoliquiritigenin, an active ingredient of Glycyrrhiza, elicits antinociceptive effects via inhibition of Na_v channels. Naunyn Schmiedebergs Arch Pharmacol 2021;394:967–80.

[14] Tomohiro D, Mizuta K, Fujita T, Nishikubo Y, Kumamoto E. Inhibition by capsaicin and its related vanilloids of compound action potentials in frog sciatic nerves. Life Sci 2013;92:368–78.

[15] Kawasaki H, Mizuta K, Fujita T, Kumamoto E. Inhibition by menthol and its related chemicals of compound action potentials in frog sciatic nerves. Life Sci 2013;92:359–67.

[16] Matsushita A, Fujita T, Ohtsubo S, Kumamoto E. Traditional Japanese medicines inhibit compound action potentials in the frog sciatic nerve. J Ethnopharmacol 2016;178:272–80.

[17] Clitherow KH, Murdoch C, Spain SG, Handler AM, Colley HE, Stie MB, et al. Mucoadhesive electrospun patch delivery of lidocaine to the oral mucosa and investigation of spatial distribution in a tissue Using MALDI-Mass Spectrometry Imaging. Mol Pharm 2019;16:3948–56.

[18] Kumamoto E. Inhibition of fast nerve conduction produced by analgesics and analgesic adjuvants—possible involvement in pain alleviation. Pharmaceuticals 2020;13:62.

[19] Okayasu I, Tachi M, Ayuse T, Wake H, Komiyama O, De Laat A. Age differences in pain sensitivity and effect of topical lidocaine on the tongue in healthy female subjects. J Oral Sci 2023:23–0167.

[20] Kumamoto E, Mizuta K, Fujita T. Peripheral nervous system in the frog as a tool to examine the regulation of the transmission of neuronal information. In: Murray JL, editor. Frogs : Biology, Ecology and Uses, New York, USA: Nova Science Publishers Inc.; 2011, p. 89–106.

[21] Ohtsubo S, Fujita T, Matsushita A, Kumamoto E. Inhibition of the compound action potentials of frog sciatic nerves by aroma oil compounds having various chemical structures. Pharmacol Res Perspect 2015;3:e00127.

[22] Cai Y, Zheng Q, Sun R, Wu J, Li X, Liu R. Recent progress in the study of Artemisiae Scopariae Herba (Yin Chen), a promising medicinal herb for liver diseases. Biomedicine and Pharmacotherapy 2020;130:110513.

[23] JASP Team. JASP (Version 0.18.1) 2023.

[24] Mase A, Makino B, Tsuchiya N, Yamamoto M, Kase Y, Takeda S, et al. Active ingredients of traditional Japanese (kampo) medicine, inchinkoto, in murine concanavalin A-induced hepatitis. J Ethnopharmacol 2010;127:742–9.

[25] Wachtel-Galor S, Benzie IFF. An introduction to its history, usage, regulation, current trends, and research needs. In: Wachtel-Galor S, Benzie IFF, editors. Herbal Medicine: Biomolecular and Clinical Aspects. Second Edition, Boca Raton, FL, USA: CRC Press; 2011, p. 1–10.

[26] Wang X, Sun W, Sun H, Lv H, Wu Z, Wang P, et al. Analysis of the constituents in the rat plasma after oral administration of Yin Chen Hao Tang by UPLC/Q-TOF-MS/MS. J Pharm Biomed Anal 2008;46:477–90.

[27] Li JY, Cao HY, Sun L, Sun RF, Wu C, Bian YQ, et al. Therapeutic mechanism of Yin-Chén-Hao decoction in hepatic diseases. World J Gastroenterol 2017;23:1125–38.

[28] Wang Y, Xing X, Cao Y, Zhao L, Sun S, Chen Y, et al. Development and application of an UHPLC-MS/MS method for comparative pharmacokinetic study of eight major bioactive components from Yin Chen Hao Tang in normal and acute liver injured rats. Evidence-Based Complementary and Alternative Medicine 2018;2018:1–12.

[29] Yu M, Zhao Y, Zhang X. Gardenoside combined with ozone inhibits the expression of P2X3 and P2X7 purine receptors in rats with sciatic nerve injury. Mol Med Rep 2018;17:7980–6.

[30] Tamaddonfard E, Tamaddonfard S, Pourbaba S. Effects of intra-fourth ventricle injection of crocin on capsaicin-induced orofacial pain in rats. Avicenna J Phytomed 2015;5:450–7.

[31] Xiao W, Li S, Wang S, Ho CT. Chemistry and bioactivity of *Gardenia jasminoides*. J Food Drug Anal 2017;25:43–61.

[32] Tao Y, Wang P, Xu X, Chen J, Huang M, Zhang W. Effects of ultrasound treatment on the morphological characteristics, structures and emulsifying properties of genipin cross-linked myofibrillar protein. Ultrason Sonochem 2023;97.

[33] Fan X, Du H, Sun Y, Jiang J, Wang Z, Yin W, et al. Suppression of the Wnt signaling pathway may contribute to the inhibition of proliferation of human hepatocellular carcinoma SMMC-7721 cells by esculetin. Oncol Lett 2017;14:1731–6.

[34] Hara K, Haranishi Y, Kataoka K, Takahashi Y, Terada T, Nakamura M, et al. Chlorogenic acid administered intrathecally alleviates mechanical and cold hyperalgesia in a rat neuropathic pain model. Eur J Pharmacol 2014;723:459–64.

[35] Bagdas D, Gul Z, Meade JA, Cam B, Cinkilic N, Gurun MS. Pharmacologic overview of chlorogenic acid and its metabolites in chronic pain and inflammation. Curr Neuropharmacol 2019;18:216–28.

[36] Rojas-Jiménez S, Pérez-Gutiérrez MS, Sánchez-Mendoza E, Martínez-Casares RM, Campos-Xolalpa N, Valladares-Cisneros MG, et al. Anti-inflammatory activity of 3, 5-diprenyl-4-

hydroxyacetophenone isolated from *Ageratina pazcuarensis*. Int J Mol Sci 2022;23:15012.

[37] Ching-Wen C, Yun-Chieh C, Yu-Chin L, Wen-Huang P. p-Hydroxyacetophenone suppresses nuclear factor-κB-related inflammation in nociceptive and inflammatory animal models. J Nat Med 2017;71:422–32.

[38] Mizuta K, Fujita T, Nakatsuka T, Kumamoto E. Inhibitory effects of opioids on compound action potentials in frog sciatic nerves and their chemical structures. Life Sci 2008;83:198–207.

[39] Catterall WA, Goldin AL, Waxman SG. International union of pharmacology. XXXIX. Compendium of voltage-gated ion channels: sodium channels. Pharmacol Rev 2003;55:575–8.

[40] Cummins TR, Sheets PL, Waxman SG. The roles of sodium channels in nociception: Implications for mechanisms of pain. Pain 2007;131:243–57.

[41] Bhattacharya A, Wickenden AD, Chaplan SR. Sodium channel blockers for the treatment of neuropathic pain. Neurotherapeutics 2009;6:663–78.

[42] Zakon HH, Jost MC, Lu Y. Expansion of voltage-dependent Na^+ channel gene family in early tetrapods coincided with the emergence of terrestriality and increased brain complexity. Mol Biol Evol 2011;28:1415–24.

[43] Widmark J, Sundstrom G, Ocampo Daza D, Larhammar D. Differential evolution of voltage-gated sodium channels in tetrapods and teleost fishes. Mol Biol Evol 2011;28:859–71.

[44] Nishino A, Okamura Y. A brief history of the Na_v channel. SEIKAGAKU 2019;91:210–23.

[45] Caldwell JH, Schaller KL, Lasher RS, Peles E, Levinson SR. Sodium channel $Na_v1.6$ is localized at nodes of Ranvier, dendrites, and synapses. Proceedings of the National Academy of Sciences 2000;97:5616–20.

[46] Black JA, Renganathan M, Waxman SG. Sodium channel $Na_v1.6$ is expressed along nonmyelinated axons and it contributes to conduction. Molecular Brain Research 2002;105:19–28.

[47] Buchanan S, Harper AA, Elliott JR. Differential effects of tetrodotoxin (TTX) and high external K^+ on A and C fibre compound action potential peaks in frog sciatic nerve. Neurosci Lett 1996;219:131–4.

[48] Kobayashi J, Ohta M, Terada Y. C fiber generates a slow Na^+ spike in the frog sciatic nerve. Neurosci Lett 1993;162:93–6.

[49] Katsuki R, Fujita T, Koga A, Liu T, Nakatsuka T, Nakashima M, et al. Tramadol, but not its major metabolite (mono-O-demethyl tramadol) depresses compound action potentials in frog

sciatic nerves. Br J Pharmacol 2006;149:319–27.

Figure legends

Fig. 1. Effects of eight Kampo medicines, used for treating oral mucositis, at 5 mg/mL on the peak amplitudes of compound action potentials (CAPs) recorded in frog sciatic nerves.

(**A**) CAP recordings in Ringer solution before Kampo medicine treatment (control; *left*), at 20 min post-exposure to eight Kampo medicines (*from upper to lower:* inchinkoto, hangeshashinto, shosaikoto, hochuekkito, juzentaihoto**,** orento, byakkokaninjinto, and orengedokuto) containing Ringer solutions with the sciatic nerve (*middle*), and in drug-free Ringer solution at 8–10 min after Kampo medicine treatment (washout; *right*). (**B**) Average time courses of changes in CAP amplitude following exposure to eight Kampo medicines at 5 mg/mL. Average CAP amplitudes of the control, recorded over 10 min at 2 min intervals: inchinkoto, 19.5 ± 5.3 mV, n = 6; hangeshashinto, 19.5 ± 3.1 mV, n = 8; shosaikoto, 18.4 ± 5.1 mV, n = 4; hochuekkito, 18.2 ± 3.1 mV, n = 5; juzentaihoto, 15.1 ± 4.6 mV, n = 5; orento, 15.7 ± 3.3 mV, n = 6; byakkokaninjinto, 20.8 ± 6.6 mV, n = 4; orengedokuto, 14.0 ± 4.4 mV, n = 4. In this and subsequent figures, each point with vertical bars represents the mean and SEM. (**C**) Summary of average CAP amplitude, relative to the control, at 20 min post-exposure to Kampo medicines. *p < 0.05 and **p < 0.01: paired Student's t-test compared with the control. ##p < 0.01: $F_{(7, 34)} = 5.44$, one-way ANOVA with the Tukey–Kramer test compared with inchinkoto.

Fig. 2. Concentration dependency for the effect of inchinkoto on frog sciatic nerve CAPs.

(**A**) CAP recordings in Ringer solution before inchinkoto treatment (control; *left*), at 20 min post-exposure to inchinkoto containing Ringer solution (1 and 10 mg/mL; *middle*), and at 8–10 min after being returned to inchinkoto-free Ringer solution (washout; *right*). (**B**) Average time courses of CAP amplitudes, relative to the control, following exposure to inchinkoto at 0.3–10 mg/mL for 20 min.

Control CAP amplitudes: 0.3 mg/mL, 32.7 ± 1.3 mV, n = 4; 1 mg/mL, 26.7 ± 5.2 mV, n = 6; 2 mg/mL, 21.7 ± 5.2 mV, n = 4; 10 mg/mL, 19.9 ± 3.5 mV, n = 6. Data for inchinkoto at 5 mg/mL were taken from Fig. 1B. (**C**) Peak CAP amplitudes, relative to the control, following treatment with inchinkoto at 0.3–10 mg/mL for 20 min, plotted against inchinkoto concentration. Concentration–response curve plotted in accordance with the Hill equation (IC_{50} = 5.4 mg/mL; Hill coefficient, n_H = −1.9). Data for inchinkoto at 5 mg/mL were taken from Fig. 1C. (**D**) Peak CAP amplitudes, relative to the control, following hangeshashinto treatment for 20 min at 1, 5, and 10 mg/mL, plotted against hangeshashinto concentration. Linear regression line is shown (R^2 = 0.468). No significant differences were observed between reductions induced by 5 and 10 mg/mL hangeshashinto ($F_{(2, 19)}$ = 6.89, $p > 0.05$; one-way ANOVA with the Tukey–Kramer test). Data for hangeshashinto at 5 mg/mL were taken from Fig. 1C.

Fig. 3. Effects of the crude drug artemisia in inchinkoto on frog sciatic nerve CAPs.

(**A**) CAP recordings before artemisia treatment (control; *left*), at 20 min post-exposure to artemisia (1, 5, and 10 mg/mL; *middle*), and at 8–10 min after being returned to artemisia-free Ringer solution (washout; *right*). (**B**) Average time courses of CAP amplitudes, relative to the control, following exposure to artemisia at 0.3–10 mg/mL for 20 min. Control CAP amplitudes: 0.3 mg/mL, 27.8 ± 1.8 mV, n = 4; 1 mg/mL, 25.7 ± 5.2 mV, n = 4; 2 mg/mL, 20.8 ± 6.1 mV, n = 3; 5 mg/mL, 15.8 ± 2.8 mV, n = 6; 10 mg/mL, 23.2 ± 4.1 mV, n = 4. (**C**) CAP peak amplitudes, relative to the control, recorded from frog sciatic nerves treated with artemisia at 0.3–10 mg/mL for 20 min, plotted against artemisia concentration. Concentration–response curve generated in accordance with the Hill equation (IC_{50} = 4.2 mg/mL; n_H = −2.3).

Fig. 4. Effects of the crude drugs rhubarb and gardenia in inchinkoto on frog sciatic nerve CAPs.

(**A**) CAP recordings before crude drugs treatment (control; *left*), at 20 min post-exposure to crude drugs (rhubarb and gardenia; each 5 mg/mL; *middle*), and at 8–10 min after being returned to drug-free Ringer solution (washout; *right*). (**B**) Average time courses of CAP peak amplitudes, relative to the control, following exposure to crude drugs (artemisia, rhubarb, and gardenia) for 20 min. Control CAP amplitudes: rhubarb, 13.1 ± 3.2 mV, n = 6; gardenia, 14.2 ± 3.4 mV, n = 6. Artemisia data were taken from Fig. 3B. (**C**) Summary of average CAP amplitude at 20 min post-exposure to artemisia, rhubarb, and gardenia (5 mg/mL each) relative to the control. *$p < 0.05$ and **$p < 0.01$: paired Student's *t*-test compared with the control. #$p < 0.05$, $F_{(2,15)} = 9.81$: one-way ANOVA with the Tukey–Kramer test compared with artemisia. Data for artemisia was taken from Fig. 3C.

Fig. 5. Effects of mixtures of crude drugs in inchinkoto on frog sciatic nerve CAPs.

(**A**) CAP recordings before crude drug mixture treatment (control; *left*), at 20 min post-exposure to crude drug mixtures (5 mg/mL artemisia + 4 mg/mL gardenia, 5 mg/mL artemisia + 1 mg/mL rhubarb, and 4 mg/mL gardenia + 1 mg/mL rhubarb; *middle*), and at 8–10 min after being returned to drug-free Ringer solution (washout; *right*). (**B**) Average time courses of CAP peak amplitudes following exposure to crude drug mixtures (artemisia, rhubarb, and gardenia) for 20 min relative to control. Control CAP amplitudes: artemisia + gardenia mixture, 11.9 ± 4.2 mV, n = 5; artemisia + rhubarb mixture, 12.7 ± 3.0 mV, n = 5; gardenia + rhubarb mixture, 8.9 ± 1.6 mV, n = 5. (**C**) Summary of average CAP amplitudes at 20 min post-exposure to three types of crude drug mixture, artemisia (5 mg/mL), and inchinkoto (10 mg/mL) relative to control. *$p < 0.05$ and **$p < 0.01$: paired Student's *t*-test compared with the control. #$p < 0.05$ and ##$p < 0.01$, compared with inchinkoto, and †$p < 0.05$, compared with artemisia: $F_{(4,22)} =$

12.08, one-way ANOVA with the Tukey–Kramer test. Data for inchinkoto and artemisia were taken from Figs. 2C and 3C, respectively.

Fig. 6. Effects of chemical compounds in artemisia on frog sciatic nerve CAPs.

(**A**) Chemical structures of the major bioactive ingredients of artemisia: scoparone, scopoletin, esculetin, chlorogenic acid (CGA), caffeic acid, 4'-hydroxyacetophenone (4'-HA), and eugenol. (**B**) CAP recordings before treatment with chemical compounds (control; *left*), at 20 min post-exposure with chemical compounds (each 1 mmol/L; *middle*), and at 8–10 min after being returned to drug-free Ringer solution (washout; *right*). (**C**) Summary of average CAP amplitude at 20 min after the beginning of exposure to seven chemical compounds (each 0.1–3 mmol/mL) relative to the control. Eugenol (1 and 3 mmol/L) significantly reduced CAP amplitude [1 mmol/L: $p < 0.05$, 45% ± 7% of the control (10.9 ± 4.2 mV), n = 5; 3 mmol/L: $p < 0.05$, 0% ± 0% (11.9 ± 4.0 mV), n = 5; paired Student's *t*-test]. *$p < 0.05$: paired Student's *t*-test compared with the control.

Table 1. List of Kampo medicines analyzed, their effects on frog sciatic nerve CAP amplitudes, and powder loss on filter paper following filtration of the Ringer solution containing the medicines (each at 5 mg/mL).

Kampo medicines	Loss of Kampo medicines (%)
Byakkokaninjinto	68.2 ± 7.6 (n = 3)
Hangeshashinto	40.3 ± 10.7 (n = 4)
Hochuekkito	60.9 ± 29.2 (n = 3)
Inchinkoto	9.8 ± 4.1 (n = 3)
Juzentaihoto	30.2 ± 8.0 (n = 3)
Orengedokuto	24.3 and 18.9 (n = 2)
Orento	54.0 and 42.2 (n = 2)
Shosaikoto	28.7 and 21.0 (n = 2)

n: number of sciatic nerves examined.

Table 2. List of crude drug components in inchinkoto and their mixtures, their effects on CAP amplitudes, and powder loss on filter paper following filtration of the Ringer solution containing these drugs.

Crude drugs	Loss of crude drugs (%)	
Artemisia (5 mg/mL)	5.8% ± 0.4%	(n = 3)
Gardenia (5 mg/mL)	22.9% and 21.3%	(n = 2)
Rhubarb (5 mg/mL)	22.7% ± 4.5%	(n = 3)
Artemisia (5 mg/mL) + Gardenia (4 mg/mL)	22.6% ± 2.0%	(n = 4)
Artemisia (5 mg/mL) + Rhubarb (1 mg/mL)	18.4% ± 1.7%	(n = 3)
Gardenia (4 mg/mL) + Rhubarb (1 mg/mL)	38.6% ± 4.9%	(n = 3)

n: number of sciatic nerves examined.

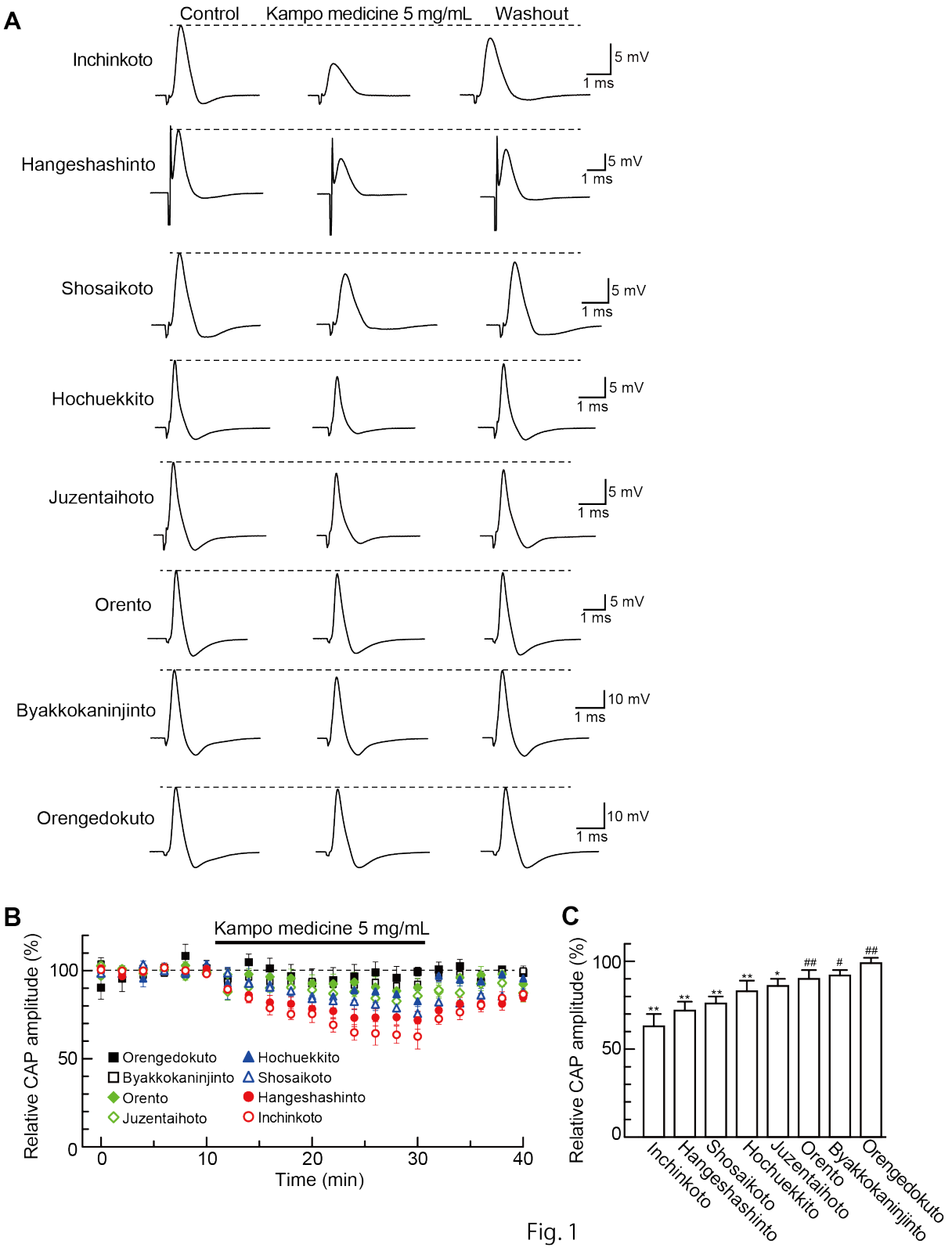
A
Control
Kampo medicine 5 mg/mL
Washout
Inchinkoto
Hangeshashinto
Shosaikoto
Hochuekkito
Juzentaihoto
Orento
Byakkokaninjinto
Orengedokuto
5 mV
10 mV
1 ms
B
Kampo medicine 5 mg/mL
Relative CAP amplitude (%)
Time (min)
Orengedokuto
Byakkokaninjinto
Orento
Juzentaihoto
Hochuekkito
Shosaikoto
Hangeshashinto
Inchinkoto
C
Relative CAP amplitude (%)
Inchinkoto
Hangeshashinto
Shosaikoto
Hochuekkito
Juzentaihoto
Orento
Byakkokaninjinto
Orengedokuto

Fig. 1

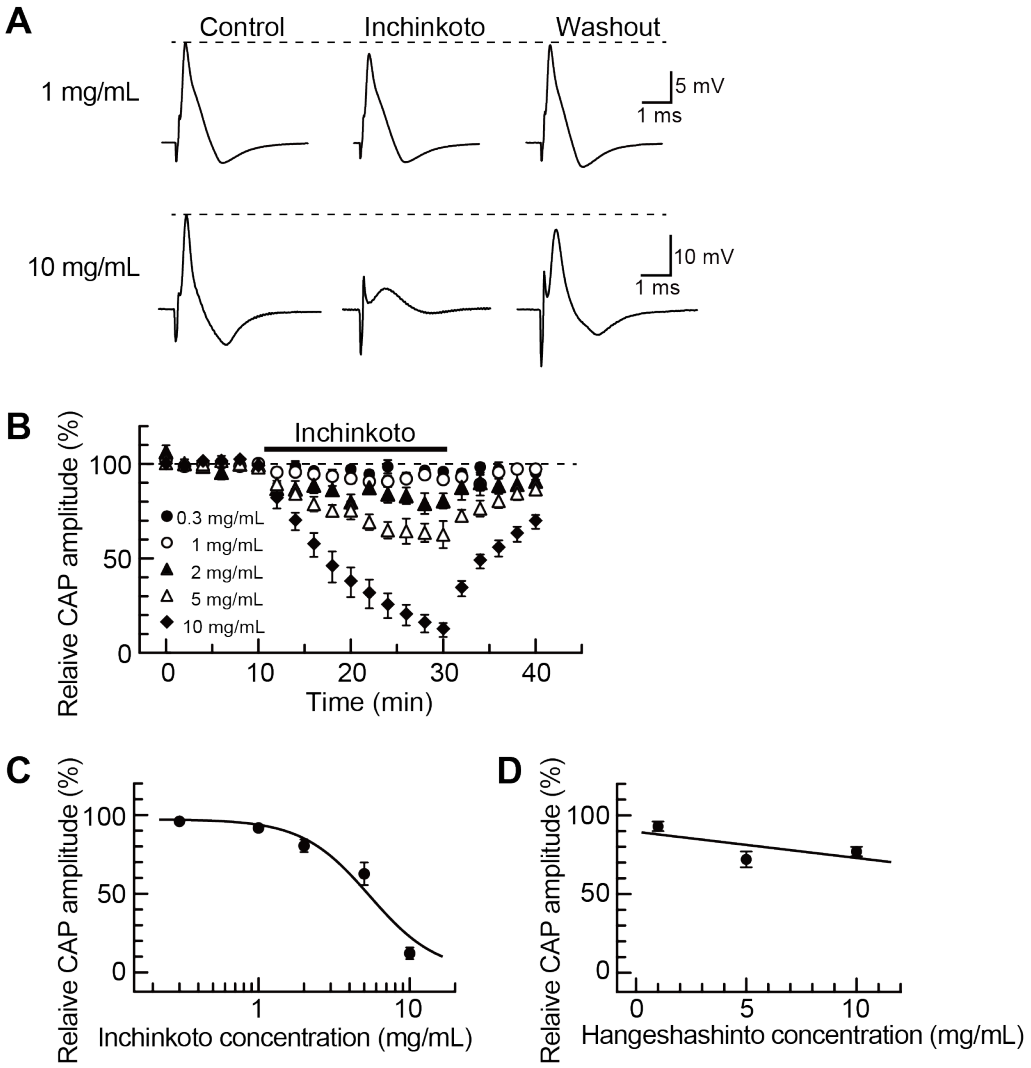
A
Control
Inchinkoto
Washout
1 mg/mL
5 mV
1 ms
10 mg/mL
10 mV
1 ms
B
Inchinkoto
Relaive CAP amplitude (%)
100
50
0
0.3 mg/mL
1 mg/mL
2 mg/mL
5 mg/mL
10 mg/mL
0
10
20
30
40
Time (min)
C
Relaive CAP amplitude (%)
100
50
0
1
10
Inchinkoto concentration (mg/mL)
D
Relaive CAP amplitude (%)
100
50
0
0
5
10
Hangeshashinto concentration (mg/mL)

Fig.2

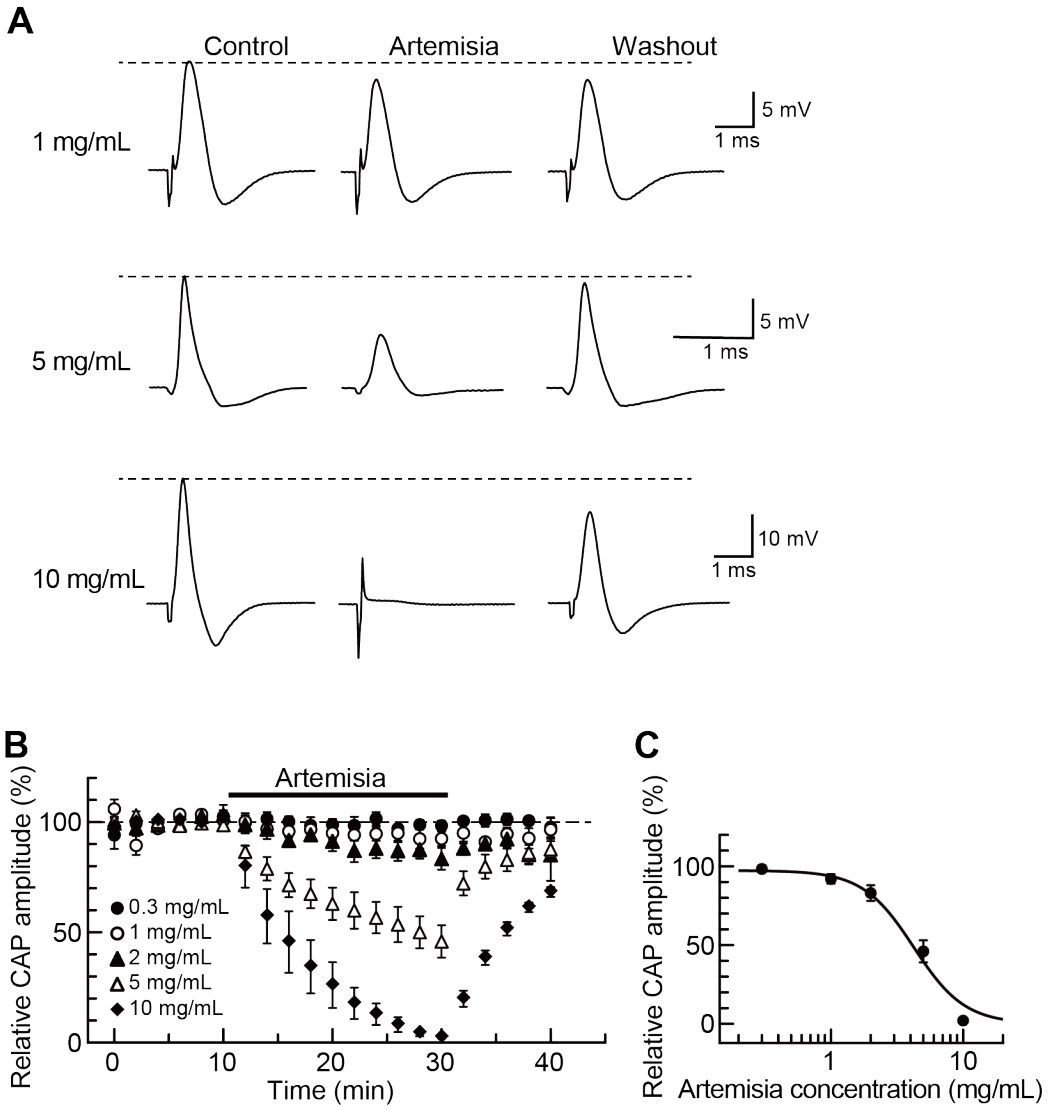
A
Control
Artemisia
Washout
1 mg/mL
5 mV
1 ms
5 mg/mL
5 mV
1 ms
10 mg/mL
10 mV
1 ms
B
Artemisia
Relative CAP amplitude (%)
100
50
0
0.3 mg/mL
1 mg/mL
2 mg/mL
5 mg/mL
10 mg/mL
0
10
20
30
40
Time (min)
C
Relative CAP amplitude (%)
100
50
0
1
10
Artemisia concentration (mg/mL)

Fig.3

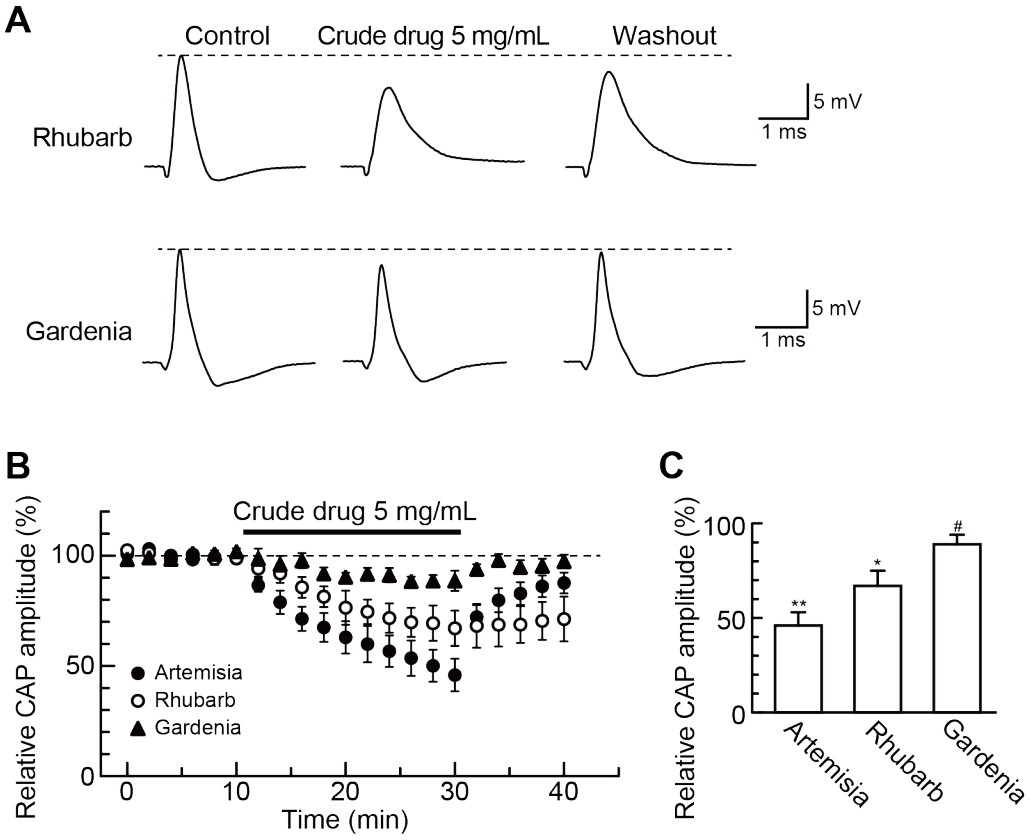
A
Control
Crude drug 5 mg/mL
Washout
Rhubarb
5 mV
1 ms
Gardenia
5 mV
1 ms
B
Crude drug 5 mg/mL
Relative CAP amplitude (%)
100
50
0
Artemisia
Rhubarb
Gardenia
0
10
20
30
40
Time (min)
C
Relative CAP amplitude (%)
100
50
0
**
*
#
Artemisia
Rhubarb
Gardenia

Fig.4

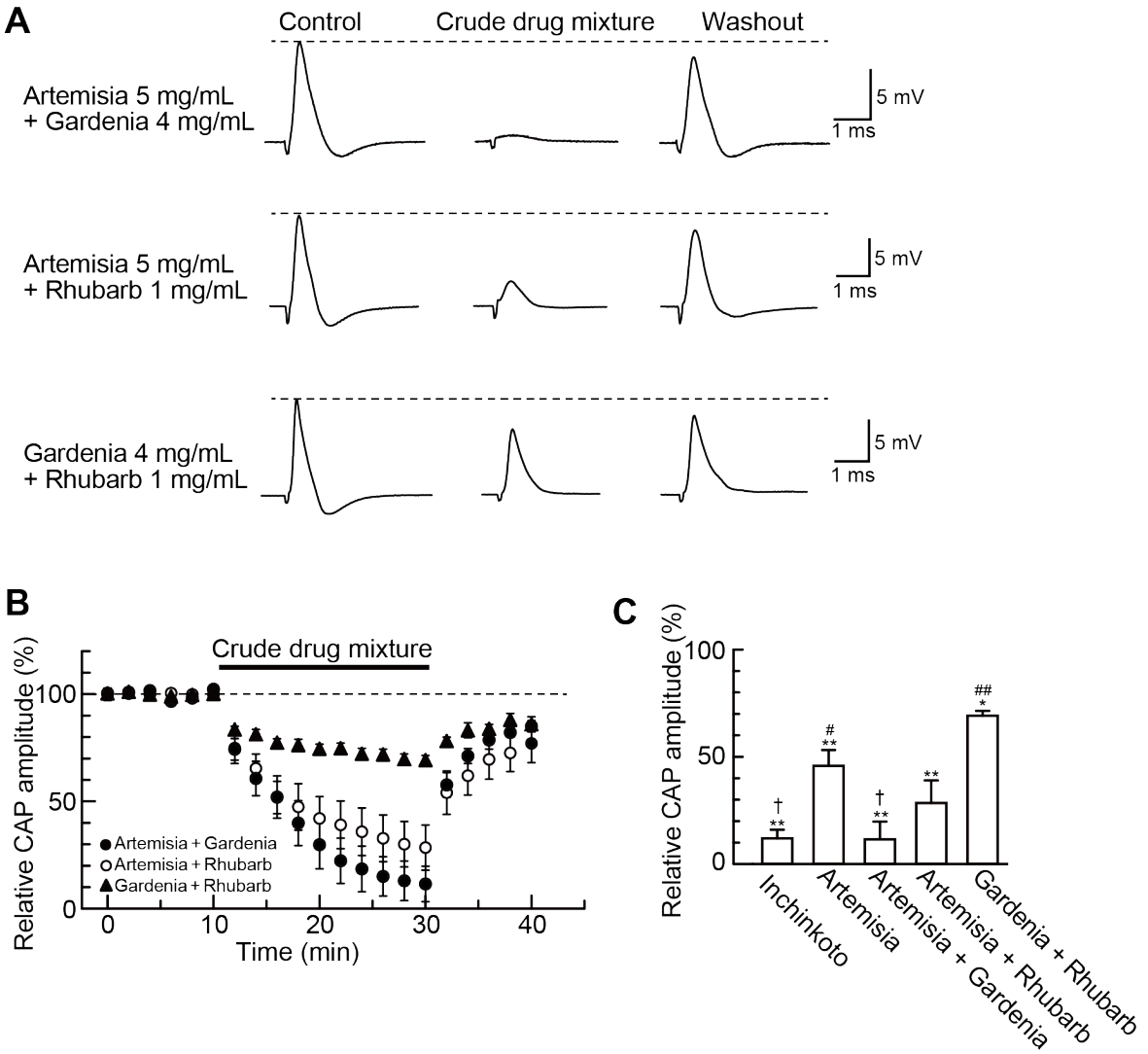
A
Control
Crude drug mixture
Washout
Artemisia 5 mg/mL
+ Gardenia 4 mg/mL
5 mV
1 ms
Artemisia 5 mg/mL
+ Rhubarb 1 mg/mL
5 mV
1 ms
Gardenia 4 mg/mL
+ Rhubarb 1 mg/mL
5 mV
1 ms
B
Crude drug mixture
Relative CAP amplitude (%)
100
50
0
Artemisia + Gardenia
Artemisia + Rhubarb
Gardenia + Rhubarb
0
10
20
30
40
Time (min)
C
Relative CAP amplitude (%)
100
50
0
†
**
#
**
†
**
**
##
*
Inchinkoto
Artemisia
Artemisia + Gardenia
Artemisia + Rhubarb
Gardenia + Rhubarb

Fig.5

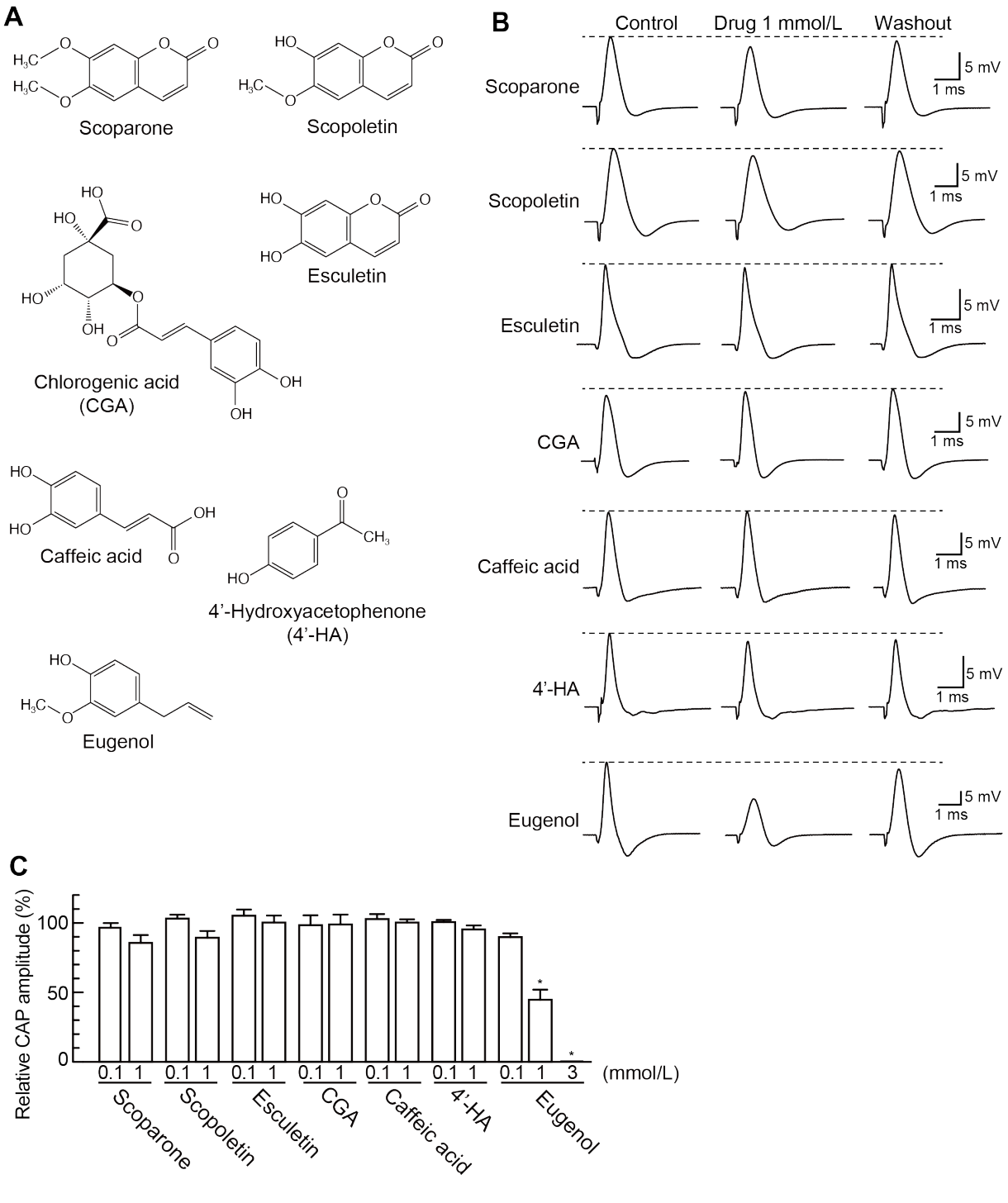
A
Scoparone
Scopoletin
Esculetin
Chlorogenic acid
(CGA)
Caffeic acid
4'-Hydroxyacetophenone
(4'-HA)
Eugenol
B
Control
Drug 1 mmol/L
Washout
Scoparone
Scopoletin
Esculetin
CGA
Caffeic acid
4'-HA
Eugenol
5 mV
1 ms
C
Relative CAP amplitude (%)
100
50
0
0.1 1
0.1 1
0.1 1
0.1 1
0.1 1
0.1 1
0.1 1 3
(mmol/L)
Scoparone
Scopoletin
Esculetin
CGA
Caffeic acid
4'-HA
Eugenol

Fig.6