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Inhibitory effects of geranium essential oil and its major component, citronellol, on degranulation and cytokine production by mast cells

Yuko Kobayashi¹, Harumi Sato¹, Mika Yorita¹, Hiroto Nakayama², Hironari Miyazato², Keiichiro Sugimoto² and Tomoko Jippo^{1,*}

¹Faculty of Human Life Sciences, Department of Food and Nutrition, Senri Kinran University, Suita, Japan;

²Research and Development Center, Nagaoka Perfumery Co., Ltd., Ibaraki, Japan

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We investigated the effects of geranium essential oil (GEO) on anaphylaxis. GEO can exert antioxidant and anti-inflammatory effects, but its roles in allergic reactions are incompletely understood. Here, we used mouse cells to show that GEO inhibited the degranulation of cultured mast cells (CMCs). Citronellol is the major component of GEO and inhibited CMC degranulation. The L-enantiomer of citronellol more effectively suppressed CMC degranulation than did D-citronellol. We also examined whether citronellol could inhibit the immunoglobulin (Ig) E-induced production of tumor necrosis factor (TNF)- α . Treatment with various concentrations of citronellol before CMC activation with IgE significantly inhibited the induction of TNF- α in a dose-dependent manner. Mechanistically, citronellol suppressed the phosphorylation of mitogen-activated protein kinase (ERK), which is critical for ERK activation and the production of inflammatory cytokines in mast cells. These findings suggest that citronellol may represent a candidate compound for the effective treatment of allergic diseases.

Key words: anti-allergic effect; citronellol; mast cell; degranulation; cytokine production

The incidence of allergic diseases, such as allergic rhinitis, atopic dermatitis, asthma, and food allergies, has recently increased in several countries.¹⁾ Mast cells and basophils play critical roles in various biological processes related to allergic diseases.^{2,3)} These cells express the high-affinity receptor for immunoglobulin (Ig)E on their cell surface. Interactions between multivalent antigens and surface-bound IgE induces the secretion of granule-stored mediators and the *de novo* synthesis of cytokines.⁴⁾ These mediators and cytokines can induce the migration of neutrophils and macrophages and reactions triggered by these cells result in

tissue inflammation and allergic reactions.⁵⁾ During allergic inflammation, activated mast cells release tumor necrosis factor (TNF)- α ,⁶⁾ which induces the expression of adhesion factors for vascular endothelial cells, white blood cell accumulation, and increased fibrosis, resulting in further inflammation.^{7–9)} Importantly, mitogen-activated protein kinase (MAPK) and transcription factors regulate cytokine production in inflammatory responses.^{10–13)}

Essential oils can be extracted from plant materials, such as flowers, leaves, peel, and seeds, and are used in aromatherapy. Importantly, they have been reported to also have antioxidant, anticancer, anti-inflammatory, and antimicrobial bioactivities.^{14–16)} Geranium is native to South Africa but is also grown in many regions worldwide. It is widely known as a medicinal herb that can be used against various diseases.¹⁷⁾ Geranium essential oil (GEO) is an important material for the cosmetic, perfume, aromatherapy, and food industries¹⁷⁾ and has also been used for many years as a traditional medicine as it has antibacterial, antioxidant, and anti-inflammatory properties.^{17–21)} However, the effects of GEO on anaphylactic reactions have not been fully characterized.

Here, we report that GEO dose-dependently inhibited the degranulation of murine-cultured mast cells (CMCs) activated by IgE. Citronellol, the major component of GEO inhibited IgE-induced degranulation and TNF- α production in CMCs. Furthermore, we showed that citronellol-inhibited phosphorylation of mitogen-activated protein kinase (ERK) in CMCs.

Materials and methods

Materials. Hydro-distilled essential oil from geranium foliage (*Pelargonium graveolens* l'Her; from a plantation in China) was purchased from Charabot (Grasse, France). Citronellol (racemate), (–)-citronellol, citronellyl formate (racemate), and geraniol were

*Corresponding author. Email: k-jippou@cs.kinran.ac.jp

Abbreviations: GEO, geranium essential oil; CMCs, cultured mast cells; TNF- α , tumor necrosis factor- α ; Ig, immunoglobulin; FBS, fetal bovine serum; ELISA, enzyme-linked immunosorbent assay; α -MEM, alpha minimum essential medium; PWM, pokeweed mitogen; PWM-SCM, pokeweed mitogen-stimulated spleen cell conditioned medium; PNAG, *p*-nitrophenyl-*N*-acetyl- β -D-glucopyranoside; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazoliumbromide; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase.

purchased from Inoue Perfumery MFG (Tokyo, Japan). (+)-citronellol, alpha minimum essential medium (α -MEM), antibiotic solution, pokeweed mitogen (PWM), *p*-nitrophenyl-*N*-acetyl- β -D-glucopyranoside (PNAG), and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazoliumbromide (MTT) were obtained from Sigma-Aldrich, Japan (Tokyo, Japan). Fetal bovine serum (FBS) was obtained from Nippon Bio-Supply Center (Nagoya, Japan). Mouse IgE, rat anti-mouse IgE, antiphospho-ERK1/2 (Thr-202/Tyr-204), anti-ERK1/2, anti-phospho-p38 (Thr-180/Tyr-182), and anti-p38 mouse IgG were obtained from BD Biosciences (Costa Mesa, CA, USA). Horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG and a mouse TNF- α enzyme-linked immunosorbent assay (ELISA) kit were purchased from Invitrogen (Carlsbad, CA, USA). Protease and phosphatase inhibitor cocktails, membrane-blocking reagent (Blocking One), and luminol-based chemiluminescence assay kits for western blotting (Chemi-Lumi One Super) were obtained from Nacalai Tesque (Kyoto, Japan).

Animals. The study protocol was approved by the Animal Care and Use Committee of Senri Kinran University (Osaka, Japan). All animal experiments were undertaken according to the Guidelines Relating to the Care and Use of Experimental Animals of the Japanese Association for Laboratory Animal Science.

Male ICR mice (9 weeks old) were purchased from Japan SLC (Hamamatsu, Japan). All animals were housed in a controlled room at $23 \pm 2^\circ\text{C}$ with $55 \pm 10\%$ humidity. Mice had access to standard laboratory rodent feed (Oriental Yeast, Tokyo, Japan) and water *ad libitum*.

Cells. Pokeweed mitogen-stimulated spleen cell-conditioned medium (PWM-SCM) was prepared according to the method described by Nakahata *et al.*²²⁾ ICR mice were used to obtain CMCs. Mice were sacrificed by decapitation after ether anesthesia, and femora were removed and flushed to isolate bone marrow cells. Bone marrow cells were cultured in α -MEM supplemented with 10% PWM-SCM and 10% heat-inactivated FBS and then half of the medium was replaced every 7 days. At 4 weeks after initiating the culture, >95% of the cells were CMCs.

Degranulation of CMCs. The release of β -hexosaminidase has been reported to correlate with the release of histamine, a major component of mast cell granules.²³⁾ Therefore, CMC degranulation can be determined using an assay to measure β -hexosaminidase release, as described previously.²⁴⁾ Briefly, CMCs were sensitized for 2 h at 37°C with IgE ($1\ \mu\text{g}/\text{mL}$). Cells were washed with Tyrode's buffer (137 mM NaCl, 5.6 mM glucose, 11.9 mM NaHCO_3 , 2.7 mM KCl, and 0.32 mM NaH_2PO_4) containing 1 mM CaCl_2 and 0.5 mM MgCl_2 . Then, various concentrations of GEO were added. Cells were stimulated for 1 h at 37°C with anti-IgE ($0.1\ \mu\text{g}/\text{mL}$) immediately after the addition of GEO. Cell supernatants and total cell

lysates were dissolved in 2% Triton X-100, harvested, and then substrate solution (PNAG in 0.1 M sodium citrate buffer, pH 4.5) was added. Each reaction was terminated after 90 min by the addition of 0.2 M glycine (pH 11.0). The amount of β -hexosaminidase in supernatants and cell lysates was quantified by spectrophotometric analyses by measuring the hydrolysis of PNAG at 405 nm. GEO was diluted to 10% (v/v) with dimethyl sulfoxide and then diluted with Tyrode's buffer (v/v). Experiments using citronellol, citronellyl formate, and geraniol used the same experimental protocol as for GEO. The percent inhibition of β -hexosaminidase release was calculated using the following formula:

Percent inhibition

$$= \frac{(\beta\text{-hexosaminidase release without sample} - \beta\text{-hexosaminidase release with sample})}{\beta\text{-hexosaminidase release without sample}} \times 100$$

Mast cell degranulation is triggered by an increased intracellular concentration of calcium ions.²⁵⁾ Therefore, citric acid, which chelates calcium ions, was used as a positive control. By adding 10 mM citric acid, the amount of β -hexosaminidase release was reduced to 10–20%.

To confirm that the sample itself did not inhibit β -hexosaminidase activity, CMCs were sonicated and this was used as a solution that contained β -hexosaminidase. Then, the sample was added, incubated for 1 h, and the activity of β -hexosaminidase was measured.

MTT assay. Cell viability was determined using the MTT assay.²⁶⁾ Briefly, 100 μL CMCs (5×10^4) were cultured in 96-well plates for 24 h after treatment with various concentrations of GEO. An aliquot of 100 μL of the MTT solution (5 mg/mL) was added, and cells were incubated for an additional 3 h at 37°C . After removing the supernatant, the insoluble formazan product was dissolved in 100 μL 0.04 N HCl in isopropyl alcohol, and the optical density measured at 570 nm. Viability was quantified based on the amount of formazan product. Viability in the absence of GEO was assigned a control value of 100%, and GEO-treated groups were presented as a percentage of this control value. When we used citronellol as a sample, the experimental protocol was similar to that used for GEO.

Gas chromatography–mass spectrometry. Gas chromatography (GC) analyses were performed with a gas chromatograph operated with a split/splitless injector (GC-2010, Shimadzu, Kyoto, Japan) under the following specific conditions: column, InertCap[®] WAX 60 m $\times$ 0.25 mm fused silica capillary column; film thickness, 0.25 μm (GL Sciences, Tokyo, Japan); temperature program, from 80°C (hold for 5 min) to 240°C (70 min) at $3^\circ\text{C}/\text{min}$ ramp; injection temperature, 240°C ; injection volume, 1.0 μL (with 0.4 μL air); inlet pressure, 176 kPa; carrier gas, He; flow rate, 1.2 mL/min; injection mode, split (50:1); flame

ionization detector (240 °C); H₂ flow, 40 mL/min; air flow, 400 mL/min; make-up flow (He), 20 mL/min; and sampling time, 40 ms. Data handling was performed using GC solution 2.32 (Shimadzu). The area (%) represents the mean value from five GC measurements.

Gas chromatography–mass spectrometry (GC–MS) analyses were performed with a gas chromatograph (6890GC, Agilent Technologies Japan, Tokyo, Japan) coupled to a mass spectrometer (5973 MSD, Agilent Technologies Japan) under the following specific conditions: column, InertCap® WAX 60 m × 0.25 mm fused silica capillary column; film thickness, 0.25 µm (GL Sciences); temperature program, from 70 °C (5 min) to 240 °C (70 min) at 3 °C/min ramp; injection temperature, 240 °C; injection volume, 1.0 µL for oils; inlet pressure, 149 kPa; carrier gas, purified He (purity ≥99.99995%) at a flow rate of 1.2 mL/min; injection mode, split (60:1); MS interface temperature, 240 °C; MS mode, electrospray ionization; detector voltage, 70 eV; and mass range, 27–400 amu. Data handling was performed using a Chemstation G1701BJ, vB.01.00J (Agilent Technologies Japan).

Oil composition analyses were performed using GC and GC–MS. Compounds were identified by comparing the mass spectrum with the retention index obtained from standard compounds. Commercially available databases (Wiley 275 and NIST 02) were also used for preliminary compound identification.

ELISA. CMCs were sensitized for 1 h at 37 °C with IgE (1 µg/mL). Cells were washed with Tyrode's buffer containing 1 mM CaCl₂ and 0.5 mM MgCl₂. Then, various concentrations of citronellol were added. Cells were stimulated for 6 h at 37 °C with anti-IgE (0.1 µg/mL). Levels of secreted cytokines in CMC culture supernatants were measured using a sandwich ELISA according to the manufacturer's protocol for the TNF-α assay. Absorption for the avidin-horseradish peroxidase color reaction was measured at 450 nm and compared with serial dilutions of mouse recombinant TNF-α protein as a standard. Data are expressed as a percentage of the mean amount of TNF-α production in the citronellol-treated group compared with the control group that lacked citronellol.

Western blotting. CMCs were sensitized for 1.5 h at 37 °C with IgE (1 µg/mL), and various concentrations of citronellol were added and incubated for 1 h in Tyrode's buffer. Cells were stimulated with anti-IgE (0.1 µg/mL) for 10 min and then briefly centrifuged. Cell pellets were lysed in 2% Triton X-100-containing phosphate-buffered saline (PBS) with protease and phosphatase inhibitors on ice for 15 min, sonicated, and centrifuged. Cell lysates were collected and protein concentrations were assessed using BCA protein assay reagent (Thermo Fisher, Waltham, MA, USA). Equivalent amounts (14 µg) of samples were separated by SDS-PAGE and blotted electrophoretically to polyvinylidene difluoride (PVDF) membranes using a Trans-blot turbo system (Bio-Rad, Hercules, CA, USA). Membranes were blocked and incubated with a primary

anti-MAPK antibody followed by a HRP-conjugated secondary antibody. Visualization of immunoreactive proteins was performed using chemiluminescence detection reagent and a Chemi Doc XRS system (Bio-Rad). Relative amounts of immunoreactivity were quantified using Quantity One image analysis software (Bio-Rad). Results are expressed as a percentage of the mean amount of immunoreactivity in the citronellol-treated group compared with the control group that did not include citronellol.

Statistical analyses. Data represent the mean ± SD of at least three independent experiments. Statistical significance was assessed using Dunnett's multiple-comparison test; *p* < 0.05 indicated statistical significance.

Results

GEO suppresses CMC degranulation. We investigated the effects of GEO on CMC degranulation to examine its anti-allergic activities. Various concentrations of GEO were added to CMCs before IgE stimulation. We found that β-hexosaminidase release was markedly and dose-dependently reduced in CMCs upon the addition of 0.005–0.02% (44–176 µg/mL) GEO (Fig. 1(A)). This suggested GEO exerted a suppressive effect on IgE-induced CMC degranulation. As a control, we showed that GEO did not inhibit the activity of β-hexosaminidase. These findings indicated that GEO exerts its anti-allergic effects by inhibiting the release of chemical mediators from CMCs. The rate of degranulation was measured when CMCs were unstimulated to determine whether GEO induced degranulation in CMCs. We detected no difference between the rate of degranulation with or without GEO in CMCs, suggesting GEO did not affect spontaneous degranulation in CMCs.

GEO does not decrease CMC viability. Next, we examined the effects of GEO on CMC viability. CMCs were incubated with GEO at 37 °C for 24 h and their viability was assessed using the MTT assay. CMC viability did not decrease after incubation with 0.001–0.02% GEO (8.8–176 µg/mL) for 24 h (Fig. 1(B)).

Identification of the chemical components of GEO.

We examined the chemical components of GEO by GC-MS. A total of 74 compounds were detected in GEO, and we identified 43 of these components, of which citronellol (39.6%), citronellyl formate (9.9%), geraniol (5.9%), isomenthone (5.4%), and isodene (3.9%) were the major components. Of note, the GEO used in this study was of Chinese origin. This is important because the chemical composition of GEO differs depending on its origins.²⁷⁾ A high amount of citronellol is a feature of the Chinese-type GEO.

Citronellol, citronellyl formate, and geraniol inhibit CMC degranulation and citronellol has no effect on CMC viability. We examined whether the three major

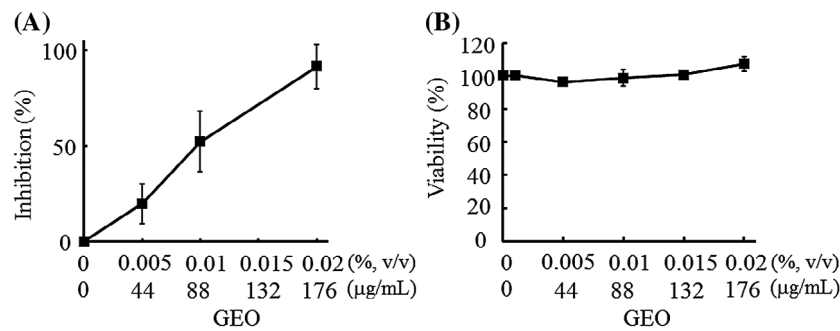


Fig. 1. Effects of GEO on degranulation, viability, and TNF- α production in CMCs.

Notes: (A) The effect of GEO on CMC degranulation. CMCs were sensitized for 2 h at 37 °C with IgE (1 $\mu\text{g/mL}$), washed with Tyrode's buffer containing 1 mM CaCl_2 and 0.5 mM MgCl_2 , and various concentrations of GEO were added. Then cells were stimulated for 1 h at 37 °C with anti-IgE (0.1 $\mu\text{g/mL}$). The degree of degranulation was calculated based on β -hexosaminidase activity in the supernatant and cell lysate. Each value represents the mean $\pm$ SD of seven experiments. (B) The effect of GEO on CMC viability. CMCs were treated with various concentrations of GEO for 24 h and cell viability was determined using the MTT assay. Each value represents the mean $\pm$ SD of three experiments.

components of GEO (citronellol, citronellyl formate, and geraniol) affected CMC degranulation. The release of β -hexosaminidase was markedly and dose-dependently decreased in CMCs following the addition of 0.25–1 mM (39–156 $\mu\text{g/mL}$) citronellol (racemate) (Fig. 2(A)). Citronellyl formate (racemate) and geraniol also inhibited CMC degranulation dose-dependently (Fig. 2(B), (C)). The inhibition of CMC degranulation by 1 mM citronellol, citronellyl formate and geraniol was 54.6, 33.8, and 32.2%, respectively. Thus, citronellol induced the greatest suppressive effects on the IgE-induced degranulation of CMCs. Next, we assessed the effects of citronellol on viability by incubating CMCs with citronellol for 24 h. The viability of CMCs did not decrease after incubation with 0.125–1 mM citronellol (Fig. 2(D)).

L-citronellol inhibits the degranulation of CMCs.

Next, we examined enantiomer of citronellol (Fig. 3) and found that 0.5 mM *L*-citronellol ((-)-citronellol) and *D*-citronellol ((+)-citronellol) inhibited degranulation of CMCs by 69.4% and 21.3%, respectively, whereas 1 mM racemic citronellol induced 54.6% inhibition. There was no difference in the viability of CMCs between 1 mM racemic citronellol and 0.5 mM *L*-citronellol (data not shown).

Citronellol inhibits IgE-induced TNF- α production in CMCs. To investigate the effects of citronellol on TNF- α production, CMCs were stimulated with IgE and their supernatants were analyzed using ELISA to detect TNF- α levels. Citronellol (0.5–1 mM)

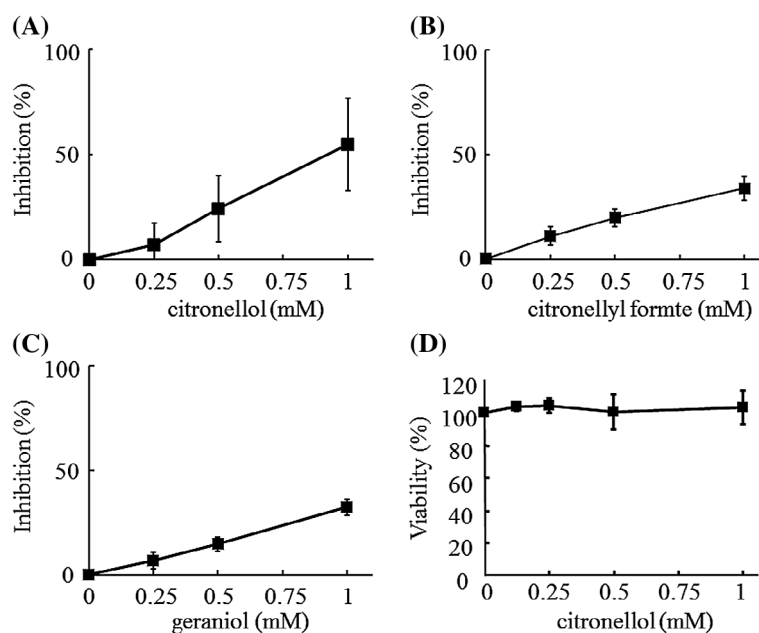


Fig. 2. Effects of citronellol, citronellyl formate, and geraniol on CMC degranulation and citronellol on CMC viability.

Notes: (A) The effect of citronellol on CMC degranulation. CMCs were sensitized for 2 h at 37 °C with IgE (1 $\mu\text{g/mL}$), washed and then various concentrations of citronellol were added. Then, cells were stimulated for 1 h at 37 °C with anti-IgE (0.1 $\mu\text{g/mL}$). The amount of degranulation was calculated based on β -hexosaminidase activity in the supernatant and cell lysate. Each value represents the mean $\pm$ SD of three experiments. (B) The effect of citronellyl formate on CMC degranulation. (C) The effect of geraniol on CMC degranulation. (D) The effect of citronellol on CMC viability. CMCs were treated with various concentrations of citronellol for 24 h. Cell viability was determined using the MTT assay. Each value represents the mean $\pm$ SD of three experiments.

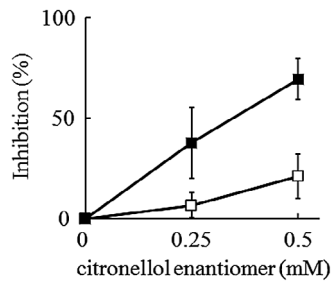


Fig. 3. Effects of citronellol enantiomers on CMC degranulation

Notes: CMCs were sensitized for 2 h at 37 °C with IgE (1 µg/mL), washed with Tyrode's buffer containing 1 mM CaCl₂ and 0.5 mM MgCl₂, and various concentrations of L-citronellol (filled squares) and D-citronellol (open squares) were added. Cells were then stimulated for 1 h at 37 °C with anti-IgE (0.1 µg/mL). The degree of degranulation was calculated based on β-hexosaminidase activity in the supernatant and cell lysate. Each value represents the mean ± SD of three experiments.

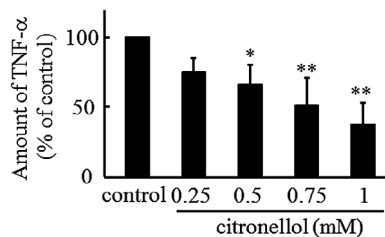


Fig. 4. Effect of citronellol on TNF-α production by CMCs.

Notes: CMCs were sensitized for 1 h at 37 °C with IgE (1 µg/mL), washed and various concentrations of citronellol were added. Then, cells were stimulated for 6 h at 37 °C with anti-IgE (0.1 µg/mL). Supernatants were analyzed using ELISA to detect levels of TNF-α. Each value represents the mean ± SD of four experiments. Comparisons between the citronellol treatment and control groups were carried out using Dunnett's multiple-comparison test; ** $p < 0.01$, * $p < 0.05$ vs. control group.

significantly inhibited IgE-induced TNF-α production ($p < 0.05$; Fig. 4).

Citronellol inhibits MAPK phosphorylation in the IgE-mediated signaling pathway. MAPKs are important components in the signaling pathways that control the synthesis and release of proinflammatory mediators

from activated mast cells during inflammatory responses.^{10,12,13} Therefore, the effect of citronellol on the phosphorylation of MAPKs was examined. Activating phosphorylation of ERK and p38 was detected after stimulation of mast cells with IgE, as shown in Fig. 5(A). Pretreatment with citronellol prior to IgE-stimulation reduced the phosphorylation of ERK, but not p38 (Fig. 5(A)) and 0.5 mM citronellol significantly inhibited ERK phosphorylation ($p < 0.05$; Fig. 5(B)).

Discussion

This study examined the anti-allergic effects of GEO. We found that GEO inhibited IgE-induced β-hexosaminidase release in CMCs. The amount of released β-hexosaminidase is commonly used to evaluate the extent of degranulation by mast cells. The release of histamine and other chemical mediators by mast cells is an important process in initiating immediate types of anaphylactic reactions.⁵ Therefore, our results indicate that GEO exerts anti-allergic effects by inhibiting the release of chemical mediators from CMCs.

The assessment of GEO on CMC viability suggested that ≤0.02% (176 µg/mL) GEO was not cytotoxic. This finding suggested that the amount of β-hexosaminidase released from CMCs was reduced after treatment with GEO, which did not affect the proliferation or viability of CMCs.

GEO is composed of various chemical constituents, including citronellol, citronellyl formate, and geraniol. Here, we showed that citronellol reduced degranulation in CMCs. The activities of citronellyl formate and geraniol were lower than that of citronellol. Additionally, we observed the amounts of citronellyl formate and geraniol in GEO were lower than that of citronellol. It has been previously reported that menthone reduced β-hexosaminidase release.²⁸ However, the amount of menthone in GEO is low. It is possible that an unknown anti-allergic component is present in GEO. Although an unknown factor may contribute to its anti-allergic activity, citronellol is most likely the major anti-allergic component of GEO.

Treatment with 1 mM racemic citronellol inhibited CMC degranulation by 54.6% while 0.5 mM L-citronel-

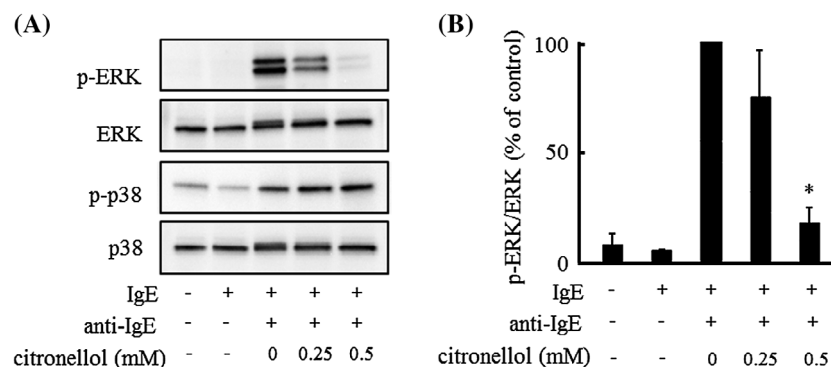


Fig. 5. Effects of citronellol on MAPK pathways.

Notes: (A) IgE-sensitized CMCs were preincubated for 1 h with or without citronellol and then were stimulated with anti-IgE for 10 min. Phosphorylated ERK and p38 were detected by immunoblotting. (B) Results from three separate measurements of ERK phosphorylation as expressed ratios (%) are shown. Each value represents the mean ± SD from three experiments. Comparisons between the citronellol treatment and control groups were made using Dunnett's multiple-comparison test; * $p < 0.05$ vs. control group.

lol caused a 69.4% inhibition. There was a small inhibitory effect on degranulation following treatment with 0.5 mM D-citronellol. Furthermore, L-citronellol did not affect the viability of CMCs. These results suggest that L-citronellol has a strong inhibitory activity on mast cell degranulation. Additionally, geranium Bourbon oil has been reported to be rich in L-citronellol.²⁹⁾ Although we could not determine the amounts of L- and D-citronellol in the GEO we used, it is possible that L-citronellol might be the major component of GEO. This requires further study.

TNF- α , a multifunctional cytokine that mediates various immune and inflammatory responses, including the recruitment, activation, and activity of T cells,^{8,30)} is produced and released from mast cells in IgE-mediated allergic reactions.⁶⁾ In the current study, citronellol inhibited TNF- α production in CMCs. The regulation of TNF- α production by citronellol might help to treat allergic disease. Furthermore, it was reported that ERK has an essential role in signal transduction pathways that mediate the production of TNF- α , IL-3, and IL-13 in mast cells,³¹⁾ and the production of TNF- α in RBL-2H3 mast cells, which requires ERK phosphorylation.¹²⁾ Based on our finding that TNF- α production and ERK activation were suppressed by citronellol, we concluded that the inhibition of TNF- α production by citronellol was highly correlated with ERK inhibition in CMCs. Moreover, MAPKs are also activated in pathways that lead to degranulation.^{12,13)} Degranulation and cytokine production are essential for allergic diseases; therefore, citronellol may exert anti-allergic activities by suppressing MAPK phosphorylation.

We demonstrated that citronellol inhibited the phosphorylation of ERK. However, phosphorylation levels of p38 were not altered in activated mast cells by pretreatment with citronellol. These results indicate that the inhibitory effect of citronellol on pro-inflammatory cytokine production is associated with ERK-signaling pathways. Similarly, luteolin and thymic immunosuppressive pentapeptide (TIPP) were reported to inhibit the phosphorylation of ERK, but not the phosphorylation of p38.^{32,33)} Lian *et al.* demonstrated that treatment with TIPP inhibited the antigen-stimulated phosphorylation of ERK, but had no effect on the activation of p38. Activation of ERK requires phosphorylation at Tyr204 and then Thr202, which is catalyzed by the dual specificity kinases MEK1/2. TIPP was shown to inhibit the activation of MEK1/2.³³⁾ This suggests that citronellol might also have an inhibitory effect on the IgE-mediated activation of CMCs via the MEK/ERK-signaling pathway. Further studies are necessary to address these issues.

We showed that citronellol has anti-allergic effects on IgE-stimulated CMCs including the inhibition of CMC degranulation. Mast cells initiate exocytosis of the contents of secretory granules in response to IgE receptor crosslinking.⁴⁾ Polyphenol-enriched apple extracts reduced mast cell degranulation by inhibiting the binding between IgE and the IgE receptor, which has anti-allergic effects.³⁴⁾ In the presence of citronellol, mast cells might not bind to IgE because of the absent or weak expression of IgE receptors. Further studies are required to elucidate the inhibitory mechanisms triggered by citronellol that alter mast cell activation.

We demonstrated that citronellol reduced degranulation, TNF- α production, and ERK phosphorylation in CMCs. This is the first study, to the best of our knowledge, to demonstrate the marked anti-allergic activity of citronellol. A previous study reported citronellol suppressed cyclo-oxygenase-2 expression and activators of peroxisome proliferator-activated receptor- α and - γ , indicating citronellol has anti-inflammatory properties.³⁵⁾

In conclusion, we showed that GEO and citronellol inhibited β -hexosaminidase release from CMCs that was induced by an IgE-mediated pathway. Citronellol also inhibited IgE-induced TNF- α production. These findings suggest citronellol exerts an inhibitory effect on allergic reactions. Furthermore, the anti-allergic effects of citronellol suggest its possible therapeutic application in the treatment of inflammatory allergic diseases.

Author contributions

Y.K. and T.J. conceived and designed the experiments. Y.K., H.S., M.Y. and T.J. performed the experiments. Y.K., K.S. and T.J. analyzed the data. H.N., H.M. and K.S. contributed the preparation and analysis of materials. Y.K. and T.J. wrote the paper. All authors have read and approved the final manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

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