Cucumis sativus extract elicits chloride secretion by stimulation of the intestinal TMEM16A ion channel

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ABSTRACT

Context: Cucumber (Cucumis sativus Linn. [Cucurbitaceae]) is widely known for its purgative, antidiabetic, antioxidant, and anticancer therapeutic potential. However, its effect on gastrointestinal (GI) disease is unrecognised.

Objective: This study investigated the effect of C. sativus fruit extract (CCE) on intestinal chloride secretion, motility, and motor function, and the role of TMEM16A chloride channels.

Materials and methods: CCE extracts were obtained from commercially available cucumber. Active fractions were then purified by HPLC and analysed by high resolution mass spectrometry. The effect of CCE on intestinal chloride secretion was investigated in human colonic T84 cells, ex vivo mouse intestinal tissue using an Ussing chamber, and the two-electrode voltage-clamp technique to record calcium sensitive TMEM16A chloride currents in Xenopus laevis oocytes. In vivo, intestinal motility was investigated using the loperamide-induced C57BL/6 constipation mouse model. Ex vivo contractility of mouse colonic smooth muscles was assessed by isometric force measurements.

Results: CCE increased the short-circuit current ($I_{sc}$ 34.47 ± 1 μA/cm²) and apical membrane chloride conductance (ΔLCl 95 ± 8.1 μA/cm²) in intestinal epithelial cells. The effect was dose-dependent, with an EC50 value of 0.06 μg/mL. CCE stimulated the endogenous TMEM16A-induced Cl- current in Xenopus laevis oocytes. Moreover, CCE increased the contractility of smooth muscle in mouse colonic tissue and enhanced small bowel transit in CCE-treated mice compared to loperamide controls. Mass spectrometry suggested a cucurbitacin-like analogue with a mass of 512.07 g/mol underlying the bioactivity of CCE.

Conclusion: A cucurbitacin-like analog present in CCE activates TMEM16A channels, which may have therapeutic potential in cystic fibrosis and intestinal hypodynamic disorders.

Introduction

Intestinal hydration primarily requires active Cl- secretion through chloride channels present in the apical membrane (Frizzell et al. 1981; Begenisich and Melvin 1998). These include the cystic fibrosis transmembrane conductance regulator (CFTR) channel and the Ca2+-activated Cl channel (CaCC). Classically, regulation of these ion channels occurs in response to agents that alter either cyclic nucleotides or intracellular calcium ([Ca2+]i), respectively (Hoque et al. 2010). Defective Cl transport by the intestinal epithelia has been described among patients with cystic fibrosis (CF). The pathogenesis of gastrointestinal (GI) disease in CF is thought to involve defective CFTR-mediated Cl secretion that results in a relatively dehydrated luminal environment. A major consequence of the altered luminal environment is the accumulation of mucus in the CF intestine that can lead to prolonged intestinal transit time, distal intestinal obstruction syndrome (DIOS) and chronic constipation (Sabharwal 2016). CF-associated constipation affects ~47% of the CF population, and unfortunately it is not widely recognised. Furthermore, gastrointestinal motility disorders are frequently reported in CF (Olivier et al. 2015). Mutations in the CF gene likely lead to uniform loss of CFTR function although the severity of the disease differs among affected organs (Boat et al. 1989). These differences are, in part, due to compensatory expression of alternative, non-CFTR, Cl- channels in the epithelium. Many studies have demonstrated that the gene expression of ANO1 (TMEM16A) in oocytes and mammalian cells leads to the expression of a calcium-activated chloride channel (CaCC) and Cl- currents (Caputo et al. 2008; Schroeder et al. 2008; Yang et al. 2008). It has been proposed that TMEM16A is the major CaCC expressed in the intestinal epithelium (Ousingsawat et al. 2009) and that this channel participates in intestinal Cl secretion (Flores et al. 2009). ANO1 transcripts and TMEM16A are expressed robustly in gastrointestinal muscles, specifically in the interstitial cells of Cajal (ICC) in murine, non-human primate, and human GI tracts (Chen et al. 2007; Hwang et al. 2009; Gomez-Pinilla et al. 2009). Normal motor activity, such as peristalsis and...
segmentation in the GI tracts, is mediated via TMEM16A in the ICC of GI smooth muscles by the generation of electrical slow wave activity (Zhu et al. 2009). Thus, therapies targeting the activation of alternative Cl\(^-\) channels like TMEM16A, have received considerable attention for alleviating intestinal CF, as well as intestinal dynamic disorders such as constipation and constipation-pre-dominant irritable bowel syndrome.

Small molecules from natural resources have shown potential for regulating CaCC mediated Cl\(^-\) secretion in intestinal epithelial cells (Zhu et al. 2020). Indeed, *Cucumis sativus* L. (Cucurbitaceae) has been used in Indian traditional medicine since ancient times. It is cultivated worldwide and a popular vegetable in tropical countries. Different parts of the plant *viz.* leaves, fruits and seeds produce several phytoconstituents with therapeutic benefits including several fatty acids, polyphenols, sterols and cucurbitacins. The latter are triterpenoid sterols that have a wide range of pharmacological actions including purgative, diuretic, anticancer, and anti-hyperglycemic activity (Mukherjee et al. 2013; Gill et al. 2010; Patil et al. 2012). In this study, we explored the bioactivity of CCE and identified a cucurbitacin-like analogue from HPLC-purified extracts. We studied the bioactivity of CCE in a number of model systems including the following: handling of intracellular Ca\(^{2+}\) in epithelial cells, activation of intestinal TMEM16A channels, active transepithelial Cl\(^-\) secretion, and GI transit time in a constipation mouse model and *ex vivo* on intestinal smooth muscle contraction. Taken together, our study demonstrates that a cucurbacin-like analogue from *C. sativus* activates TMEM16A channels, stimulates intestinal Cl\(^-\) secretion, and augments muscle contractility and motor function. These actions imply possible utility for alleviating intestinal and intestinal hypodynamic disorders including that observed in CF.

**Materials and methods**

**Reagents**

Unless otherwise stated, all chemicals used in this study were obtained from Sigma-Aldrich. Cell culture media and foetal bovine serum (FBS) were purchased from Cell Clone and HiMedia, respectively. Penicillin-streptomycin was obtained from Invitrogen and CaCCinh-A01 was purchased from Calbiochem.

**Extract preparation and HPLC purification**

Aqueous extracts were obtained by removing the skin of commercially available cucumbers (approx. weight 500 g cucumbers) from a local market and mashing the remaining matter. The crude extracts were filtered using a Buchner funnel with Whatman grade 1 filter and the filtrates were centrifuged at 10,000 g for 10 min at 4°C. The supernatants were lyophilised and stored at −20°C. The rate of extraction of crude CCE is 1.8–2.5 g/L of filtered aqueous extract. Further purification was performed by preparative reverse phase-high performance liquid chromatography (RP-HPLC) by dissolving it into acetonitrile-water (5:95 v/v). The extract was passed through a (2.2 cm × 25 cm; 5 μm) Vydac 218TP1022 reversed phase C18 column (Waters Corporation, Milford, MA) equilibrated with buffer A (0.1% trifluoroacetic acid, HPLC grade water, Merck) in a Breeze 2 HPLC system. A flow rate of 8 mL/min was maintained and the elute was monitored using a photo diode array detector. The separation was carried out using 100% buffer A for 8 min, followed by a linear gradient, 0–60% buffer B (0.05% trifluoroacetic acid, 100% acetonitrile, Merck), for 30 min; column temperature was 20°C. The fraction containing the most prominent UV absorbing peaks was collected separately and concentrated by a Savant speed vac concentrator. The dried materials were dissolved in dimethyl sulfoxide (DMSO) to perform functional studies. For further investigation, a mass analysis was performed using a Xevo G2-XS QToF high resolution mass spectrometer (HRMS) in positive ion resolution mode controlled by MassLynx v4.1 software (Waters Corporation). Key parameters were the capillary voltage set to 2.5 kV, sampling cone set to 80 V, source offset set to 80 V, source temperature at 120°C and desolvation gas flow set to 800 L/h.

**Cell culture**

Human colonic T84 cells were routinely maintained in a ratio of Dulbecco’s modified Eagle’s medium (DMEM) and Ham’s F-12 medium supplemented with 10% foetal bovine serum (FBS), 100 units/mL penicillin, and 100 μg/mL streptomycin. T84 cells between passages 8 and 20 were seeded onto polycarbonate membrane, 12 mm Snapwell permeable support cell culture inserts (0.4 μm pore size; Costar), and grown for 10–15 days during which the media were changed every 48 h. Monolayer resistance was determined using an EVOM ohmmeter with STX2 electrodes (World Precision Instruments, Inc.) (Sheikh et al. 2013). IB3-1 cells were obtained from the American Type Culture Collection (Manassas, VA). The IB3-1 cell line is a compound heterozygote bronchial epithelial cell line from a CF patient containing one ΔF508 allele and one W1282× nonsense mutation allele. IB3-1 cells were cultured in LHC-8 medium (Invitrogen, Carlsbad, CA) supplemented with 5% FBS, 100 U/mL penicillin, 100 μg/mL streptomycin, 2 mM L-glutamine, and 1 μg/mL amphotericin B (Fungizone).

**Animal and tissue preparation**

Three to four C57BL/6 male mice, 6–8 weeks of age, and fasted overnight, were used for each group in all intestinal Cl\(^-\) secretion, charcoal transit, and smooth muscle contraction experiments. Animal experiments and protocols were approved by the Institutional Animal Ethics Committee (NICED/CPCSEA/AW/225/IAEC-KMH/3). Following euthanasia by CO2 inhalation, the mouse colonic tissues were removed, and sero-musculature strip-ping was done on the top of an ice-cold glass plate. The mucosa was typically mounted on the snap well chambers (catalog number P2304; area 0.30 cm\(^2\); Physiologic Instruments Inc.) and transepithelial transport of ions was measured (Aoun et al. 2016).

**Transepithelial I\(_{sc}\) measurement**

Monolayers were considered polarised and appropriately mounted in an Ussing chamber when resistance was equal to or greater than 1,500Ω.cm\(^2\). The T84 cells grown on Snapwell inserts were mounted in an Ussing chamber for short circuit current (Isc) measurements, which were done at 37°C with both sides of the monolayer immersed in an oxygenated HCO\(_3\)-free solution containing (in mM) 140 NaCl, 5 KCl, 1 MgSO\(_4\), 2 CaCl\(_2\), 10 HEPES, and 10 glucose, pH 7.4. Fluid (5 mL) in each half of the chamber was connected via KCl agar bridges to voltage and current electrodes and clamped at 0 mV using a VCC MC6 multi-channel voltage-current clamp amplifier (Physiologic...
Instruments). Tetrodotoxin (TTX, 0.5 μM) was used to eliminate the possible neuronal influence on short circuit currents (Isc) and amiloride (10 μM) was used to inhibit epithelial sodium channels (ENaC) (Sheikh et al. 2013). The change in Isc induced by the treatment was expressed as the difference from the baseline to the steady state. The effects of CCE on apical membrane Cl- conductance (ICl) were assessed in T84 cell monolayers after permeabilization of the basolateral membrane with 50 μg/mL nystatin and the establishment of a basolateral to apical Cl- concentration gradient according to our previously published protocol (Hoque et al. 2010).

**Two electrode voltage clamp (TEVC)**

Two electrode voltage clamp recordings from *Xenopus laevis* oocytes were performed at room temperature in standard ND-96 Ringer’s solution (in mM: 96 NaCl, 2 KCl, 1 MgCl2, 1.8 CaCl2, 5 HEPES, pH 7.5) (Woodward et al. 2010). To increase the conductance of endogenous TMEM16A currents, oocytes were treated with HPLC-purified CCE in the ND-96 bath solution. A holding potential of −40 mV was used on all oocytes.

**Intracellular calcium measurement**

IB3-1 cells were seeded on a collagen-coated cover glass. Cells were rinsed with non-supplemented media, then loaded with Fura-2/AM (Invitrogen, USA) and bathed in a Ca2+-free solution (in mM: 120 NaCl, 4.5 KCl, 2 MgCl2, 10 HEPES, pH 7.4). Images were acquired on a Zeiss microscope (Zeiss Observer A1, Germany) and a FluorArc was used to excite the cells at 340 and 380 nm, controlled via a Sutter (USA) Lambda 10-2 controller and filter wheel assembly, and the emission measured at 510 nm. All images were acquired using a CoolSnap CF CCD camera (Photometrics, USA) and fluorescence at each wavelength measured once every 5 sec. Image acquisition, analysis, and wheel control were performed by IPLab Software (BD Biosciences, USA). Cells were treated with 0.12 μg/mL of HPLC fraction of CCE in ringer’s solution with 3–5 mM CaCl2. Each data point was normalised to the individual initial fluorescence value, ΔF/F0 = (F340/F380)/(F340/F380initial) to bring all the response curves to the same pre-treatment starting point (Woodward et al. 2010).

**Intestinal motility measurements**

Constipation was induced in C57BL/6 mice through oral administration of 3 mg/kg loperamide hydrochloride daily for 5 to 6 days. One group of loperamide-treated mice was administered with HPLC-purified CCE (5 mg/kg, orally). The control animals were administered only phosphate buffer solutions (PBS). Animals were fasted for 24 h before the experiment and divided into three groups administered respectively with PBS, loperamide, and loperamide plus CCE. After 15 min of CCE administration, the animals were fed 0.3 mL of charcoal meal (consisting of 10% vegetable charcoal in 5% gum acacia). After 30 min of charcoal administration, the animals were sacrificed by cervical dislocation and the total intestines were isolated. The charcoal transit ratio was calculated as the percentage of distance transited by the charcoal relative to the total length of the small intestine (pyloric sphincter to the cecum).

Muscle contraction

Muscle contraction was measured by an isometric force transducer (Grass Technologies, West Warwick, RI). The colon segment (~2 cm) of adult mice was carefully removed and tied with a silk thread to each end to hang vertically into an organ bath containing Krebs solution containing (in mM): 120 NaCl, 4.2 KCl, 1.2 MgSO4, 0.6 KH2PO4, 25 NaHCO3, 11.1 glucose, and 1.8 CaCl2 (pH 7.4). The organ bath was aerated with 95% O2, 5% CO2; temperature was maintained at 37°C for the duration of the experiment. After a stable contraction was obtained, experiments were performed in the presence of carbachol (CCH) and purified CCE fraction. The effect of CaCCinhA01 (TMEM16A inhibitor) on tension evoked by CCE was also evaluated. All contractions were recorded using AcqKnowledge data acquisition software version 3.9.1 (Biopac Systems, Goleta, CA) (Forrest et al. 2012).

**Statistical analysis**

Statistical data and graphic analysis were performed using Origin 6.0 (OriginLab, Northampton, MA, USA) software. Data are expressed as mean ± standard error (SE). Statistical significance was determined by using paired or unpaired t-test, multiple t-test corrected with the Holm-Sidak method, and ANOVA as applicable. A p value <0.05 was considered statistically significant as indicated.

**Results**

**Activation of Cl- secretion by C. sativus extract (CCE) in human colonic T84 cells**

In CF, TMEM16A-mediated secretion is intact and provides a therapeutic target to circumvent Cl- secretion defects. Thus, the discovery of novel compounds that would stimulate TMEM16A without adverse effects could compensate for the loss of Cl- secretion and improve the adverse phenotype associated with CF. We performed bioactivity analysis of crude CCE by measuring transepithelial Isc in T84 monolayers in Ussing chamber. Crude CCE produced a dose-dependent increase in Isc when applied on the apical side with a maximal effect seen at a concentration of 0.4 mg/mL (Figure 1(A)). This concentration of the crude CCE was chosen in the succeeding experiments to monitor the impact of various interventions. In contrast, basolateral addition of CCE induced a negligible increase in Isc. After addition of apical CCE, cells were further treated with the adenylate cyclase activators forskolin (FSK), 10 μM) to activate CFTR mediated Cl- secretion. A steady state response (cAMP mediated Cl- secretion) was determined by using paired or unpaired t-test, multiple t-test corrected with the Holm-Sidak method, and ANOVA as applicable. A p value <0.05 was considered statistically significant as indicated.
CFTRinh-172 (Figure 1(D)), suggesting that CCE targets another Cl- conductance. To further determine whether the increase in $I_{sc}$ was reversible, CCE was washed out from the apical chamber and $I_{sc}$ gradually returned to near baseline level; a second application of CCE still triggered a current (Figure 1(E)), albeit less pronounced (25% recovery), suggesting that the effect was reversible and repeatable. These observations strongly support the assertion that crude CCE activated a CFTR-independent chloride secretion via an interaction with a "receptor" present on the luminal membrane.

**Bioactivity-directed fractionation of CCE by HPLC**

Crude CCE was fractionated further by RP-HPLC using a water-acetonitrile gradient to obtain discrete compounds present in the aqueous extract. Four prominent ultraviolet absorbing fractions were obtained and collected (Figure 2(A)). Functional screening of these four fractions revealed that fraction #2 with a retention time of 26 min, evoked an increase in transepithelial Cl- current in nystatin-permeabilized T84 monolayers (Figure 2(B)). The stimulatory effect of this fraction (HPLC-purified CCE) was dose-dependent, with a threshold concentration of 0.04 μg/mL and a maximal effect observed at 0.12 μg/mL (Figure 2(B)). These responses were completely inhibited by DIDS (4,4'-disothiocyanato-stilbene-2,2'-disulfonic acid), a broad spectrum Ca$^{2+}$ activated Cl- channel blocker that is known to inhibit TMEM16A. A higher concentration of the fraction (0.16 μg/mL) failed to activate the Cl- current further. Analysis shows that the EC50 of this effect was 0.06 μg/mL (Figure 2(B) inset). The relative activity of the HPLC-purified CCE (0.12 μg/mL) was ~3000-fold greater than that produced by the crude (0.4 mg/mL) fraction. The LC-MS spectra of the HPLC-purified CCE showed 7 prominent protonated molecular ions ranging from 175–533 m/z. Among these ions the most abundant species were seen at 203 and 513 m/z with the latter having an exact monoisotopic mass ($M^+ + H^+$) of 513.0740 m/z (Figure 2(C)). We suggest that one of the 7 prominent ions was likely responsible for the bioactivity in intestinal epithelia. Based on the abundance of molecular ions in scans of the bioactive fraction #2, the closest match for the 513 m/z ion in PubChem was for Compound CID 58599137 i.e., (9R,13R,14S,16R)-2,16-dihydroxy-17-[(E,2R)-2-hydroxy-6,6-dimethyl-3-oxohept-4-en-2-yl]-4,4,9,13,14-pentamethyl-8,10,12,15,16,17-hexahydro-7H cyclopenta[a]phenanthrene-3,11-dione having an elemental formula of C31H44O6 (Figure 2(D)).

**Activation of TMEM16A by bio-active molecules of CCE**

To examine the effect of bio-active molecule from HPLC fractions on TMEM16A, we took advantage of the robust endogenous expression of this channel in *Xenopus laevis* oocytes. Using the two-electrode voltage clamp technique, oocytes were held at -40 mV and then subjected to +20 mV voltage clamp steps.

![Figure 1](image-url)
ranging from $-100$ to $+60$ mV. Only small voltage- and time-independent leak currents were recorded in the absence of the CCE (Figure 3(A), left traces). The purified CCE (0.12 mg/mL) activated a robust membrane current exhibiting outward rectification and amplitudes consistent with the well-characterized endogenous TMEM16A-induced Cl$^-$ current in *Xenopus laevis* oocytes (Figure 3(A), right traces and Figure 3(B)). The activation of the TMEM16A by the purified CCE was transient, as evidenced by the time-dependent decline of the outward current at $+60$ mV and the instantaneous tail current upon return to $-40$ mV (Figure 3(C)). To discern the involvement of intracellular Ca$^{2+}$ on CCE-mediated Cl$^-$ channel activation, we measured intracellular Ca$^{2+}$ levels in the IB-3 cell line derived from a cystic fibrosis patient by monitoring fluorescence intensity using the ratiometric dye Fura-2 in the presence of the purified fraction. As shown in Figure 3(D), the cells were preincubated in Ca$^{2+}$-free medium and the purified fraction of CCE caused a rapid and transient rise of intracellular Ca$^{2+}$ that stabilised to a plateau level that was significantly higher than baseline. These results suggest that CCE activates TMEM16A, most likely by releasing Ca$^{2+}$ from internal stores, and that this mechanism probably underlies the ability of TMEM16A to enhance Cl$^-$ secretion.

**CCE induces intestinal contractility and motor function**

Because TMEM16A channels can promote pacemaker activity that augments smooth muscle contraction in the gastrointestinal tract (Hwang et al. 2009), experiments were performed *in vivo* to explicate the stimulatory effect of the bio-active material on the spontaneous contractility of colonic smooth muscles. Similar to its effects on cultured GI epithelial cells, Figure 4(A) shows that the purified fraction of CCE was also able to trigger Isc in mouse colonic tissue. CCH (50 μM) increased the frequency of
spontaneous phasic contractions in intact mouse colonic strips, an effect that disappeared after washout. Application of the purified fraction of CCE produced a similar effect to CCH on contractions, which was abolished by exposure to the TMEM16A inhibitor CaCCinh-A01 (10 μM) (Figure 4(B)). The motor function of upper GI tracts was estimated by small bowel transit in vivo by determining the charcoal transit. For these experiments, the loperamide-treated constipation mouse model was used because loperamide is known to inhibit colonic smooth muscle contractions, delay intestinal luminal transit, and promote constipation (Kojima et al. 2009). As shown in Figure 4(C), CCE caused a significant enhancement of the travelled distances of luminal charcoal in comparison to control mice only treated with loperamide (loperamide control: 44 ± 6.24 vs. CCE: 67.33 ± 4.63, p < .05); in fact, the travelled distance of luminal charcoal was similar to that measured in mice unexposed to loperamide. Together, these findings indicate that purified CCE may offer a beneficial role in augmenting spontaneous motility and contractility by activating TMEM16A channels.

**Discussion**

Plants produce diverse compounds and are an important source for promising drug candidates with highly selective biological activities. The primary purpose of this study was to attempt to rescue intestinal Cl− secretion, GI motility and motor function via the activation of TMEM16A channels by a natural compound that may be useful for the treatment of CF-related intestinal phenotypes.

In this study, we detected bioactivity in crude as well as HPLC fractions derived from an aqueous fruit extract of *C. sativus*. The bioactive material is a potent intracellular Ca2+ mobilizing agonist that activated TMEM16A channels. This is supported by our finding that the purified HPLC fraction activated the characteristic outwardly rectifying TMEM16A Cl− current in *Xenopus laevis* oocytes (Callamaras and Parker 2000). In human bronchial epithelial cells (IB3-1) with CCE and without (n = 3 cover slips and 32 cells for each) in zero Ca2+ bath using Fura-2. ΔF/F is the measured F340/F380 ratio normalized to the initial F measurement (see methods). CCE significantly increased intracellular Ca2+ as determined by Two-way ANOVA, p < 0.0001).
of TMEM16A, but instead involves pathways that raise $[Ca^{2+}]_i$, as a result of a G-protein coupled receptor (GPCR) activation on the luminal membrane.

Members of the Cucurbitacea family are noted for their beneficial laxative effect and also the potential for toxicity mediated by their cucurbitacin (CCB) content (Gry et al. 2006). Their beneficial laxative action is ascribed typically in the lay literature to their high-water content but may be mediated also by other factors that stimulate GI contractility including CCBs. Over 19 CCBs and numerous structurally CCB-like entities have been described and some (CCB-A, B, D, E, I) as well as their analogues (e.g., hemslecin A) are available in pure form commercially. Accordingly, we expected to find one or more of the known CCBs in the bioactive fraction we obtained from CCE. Most known CCBs and CCB-related entities have in common the classic four ring member steroid nucleus with various additions to the A and/or the D rings that lead to variations in molecular weights ranging from 344.4 to 626.6 g/mole. In addition, they have a complex stereochemistry that is not easily elucidated by mass spectrometry. Of relevance to the present work, there are numerous known CCBs in the mass range of 514 to 518 g/mol and we were surprised that, among these and related CCBs, none have molecular weights that match any of the more interesting prominent molecular ions ($m/z = 435, 484, 497, 513, 514, 533$) we observed in the bioactive fraction obtained from CCE. The authors declare they have no financial interest or competing interests, by generating electrical slow waves (Gomez-Pinilla et al. 2009). This raises the important question as to whether the CCE-induced rise in $[Ca^{2+}]_i$, could translate into TMEM16A stimulation in the ICC that would be beneficial in the treatment of CF patients such as constipation and dysmotility. We observed that CCE accelerated intestinal transit by 53% in the loperamide-treated constipation mouse model compared to loperamide alone (Figure 4(C)). Our results further showed that the bio-active material activated intestinal smooth muscle contraction in isolated mouse colon as shown in Figure 4(B). Together, these data support the role of TMEM16A channels in the ICC and its activation by our novel bioactive material. Further, our results imply that the bioactive compound may be of therapeutic benefit in CF by stimulating TMEM16A channels, enhancing transepithelial ion flux, water movement, and GI motility that collectively should improve bowel transit time.

Conclusions

This study detected a novel bio-active compound from fruit extract of *C. sativus* that increases intracellular $Ca^{2+}$ and activates TMEM16A mediated Cl$^-$ secretion and thereby may rescue defective intestinal Cl$^-$ secretion and GI motility. We suggest that CCB-like entities may be useful lead compounds for the development of novel, orally active, therapeutic agents for CF-related as well as unrelated intestinal disorders.

Disclosure statement

The authors declare they have no financial interest or competing interest.
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