

# Peppermint and caraway oils have muscle inhibitory and pro-secretory activity in the human intestine in vitro

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## Abstract

**Background:** Herbal medicinal products with a broad activity spectrum may be promising alternatives to treat functional gastrointestinal disorders (FGD). Menthacarin<sup>®</sup> is a drug with a fixed combination of peppermint and caraway oils, which is clinically used to treat FGD-associated symptoms.

**Materials:** We studied the effects of peppermint and caraway oils on contractile and secretory activity in 255 human small and large intestinal preparations derived from surgical resections (73 patients). Motility was recorded in circular smooth muscle strips and secretion with the Ussing chamber-voltage clamp technique. Electrical field stimulation evoked nerve induced contractile responses.

**Key Results:** Peppermint and caraway oil concentrations dependently inhibited muscle contractility as indicated by sustained muscle relaxation and decrease in phasic contractility. These effects occurred in small and large intestinal preparations with IC<sub>50</sub> values ranging between 17 and 90 µg/mL for peppermint oil and between 7 and 127 µg/mL for caraway oil. Neither peppermint nor caraway oil influenced the nerve evoked contractile response. The inhibition of contractile activity, but not the muscle relaxation, was prevented by the L-type calcium channel activator Bay K8644 but not by the neurotoxin tetrodotoxin. Both peppermint oil and caraway oil increased epithelial secretion, which remained in tetrodotoxin.

**Conclusion & Interference:** The findings revealed a strong muscle inhibitory and pro-secretory action of peppermint and caraway oils at clinically relevant concentrations. Both actions were nerve-independent. The inhibition of contractility was mediated by inhibition of L-type calcium channels. The effects on muscle and epithelial activity may contribute to the beneficial effects observed in patients with FGD.

## KEYWORDS

caraway oil, contraction, human intestine, Menthacarin<sup>®</sup>, peppermint oil, secretion

## 1 | INTRODUCTION

Peppermint and caraway oils are constituents of Menthacarin®. This herbal medicine targets bloating, visceral cramps, fullness and pain and all symptoms which occur in patients with FDG. The over the counter drug is used to treat functional dyspepsia (FD) <sup>1</sup> and FD patients with irritable bowel syndrome symptoms.<sup>2</sup> Guidelines recommend peppermint oil containing medications for IBS treatment.<sup>3</sup> Menthacarin® is taken as an acid-resistant coated capsule assuring that its active components—peppermint and caraway oils—are released after the passage through the stomach. It therefore exerts its effect in the small intestine and large intestine.

In an *in vitro* study Menthol (0.1–30 mmol/L), which is a major constituent of peppermint oil, reduced the amplitude of spontaneous contractions of human circular muscle strips from the colon region without affecting contractile frequency or resting muscle tone.<sup>4</sup> The same study reported that 1–30 mmol/L menthol also reduced nerve mediated and carbachol-induced contractions. The effects were blocked by the L-type calcium channel blocker nifedipine but not by the nerve blocker tetrodotoxin. This spasmolytic action may partly explain the clinical benefit of peppermint oil.<sup>5</sup> In the stomach of healthy volunteers, peppermint oil decreased motility index in the fasting but not fed state but had no effect on gastric sensitivity to distension, basal muscle tone, or fundus accommodation.<sup>6</sup> Spasmolytic effects also occurred in the duodenum of healthy volunteers after application of peppermint and caraway oils at doses present in Menthacarin®.<sup>7</sup>

Data on the effects of caraway oil or its constituents on muscle activity in the intestine are not available. However, caraway fruit extract stimulated motility in the guinea pig proximal and distal stomach, whereas peppermint leaf extract had a very inconsistent effect.<sup>8</sup> While peppermint leaf extract had a potent pro-secretory action through activation of chloride channels in the human intestine, caraway fruit extract did not affect epithelial ion fluxes.<sup>9</sup>

To the best of our knowledge, there is no comprehensive study on the effects and mode of action of peppermint and caraway oils in human small and large intestinal muscle strips or epithelial preparations. It was therefore the aim of this project to study the effects of peppermint and caraway oils individually as well as in combination on muscle contractions and epithelial Cl<sup>-</sup> secretion in isolated human intestinal preparations.

## 2 | MATERIALS AND METHODS

### 2.1 | Human tissue

All experiments were performed on human small and large intestinal preparations. These were surgical resections derived from patients who underwent surgery at the Departments of Surgery of the Klinikum Freising, Rechts der Isar Munich and Klinikum Erding. The samples came from macroscopically unaffected areas as determined by the pathologists. The procedures and the use of the resections were approved by the ethic committee of the

### Key Points

Peppermint and caraway oils are widely used as alternative medical treatment of symptoms associated with functional gut disorder. We aimed to reveal their effects on human motility and secretion *in vitro*.

Both oils had muscle inhibitory and pro-secretory effects which were due to direct inhibition of smooth muscle and direct activation of epithelium.

The effects on muscle and epithelial activity may contribute to the beneficial effects observed in patients with functional gut disorders.

Klinikum Rechts der Isar (1748/07 and 2595/09) and with the informed patient's consent. Experiments were performed in accordance with the Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects). For the muscle strip studies, we studied 47 small intestinal and 192 large intestinal preparations from 67 patients (32 male and 35 female). In the result section, we describe the effects of peppermint and caraway oils on small and large intestinal samples. The average age of the patients was 65 years with a range of 46–87 years. The reasons for the surgeries were as follows: Crohn's disease, diverticulitis or carcinoma of the stomach, pancreas or colon. We pooled the data from duodenum, jejunum, and ileum, which represent the small intestine. Likewise, all colonic regions were pooled to represent the large intestine. We measured effects on epithelial secretion in 16 colonic preparations from six patients (three male and three female; average age of 62 ranging from 38 to 81). The reasons for surgery were carcinoma and diverticulitis.

The use of tissues from such a variety of diseases was possible, as we have shown that the disease per se had no negative influence on the responsiveness of the unaffected gut region.<sup>10,11</sup>

Immediately after resection, samples were transferred by fast courier service to the laboratory under aseptic conditions in cold oxygenated sterile Krebs buffer. The Krebs solution contained (in mmol/L) 117 NaCl, 4.7 KCl, 1.2 MgCl<sub>2</sub>, 1.2 NaH<sub>2</sub>PO<sub>4</sub>, 25 NaHCO<sub>3</sub>, 2.5 CaCl<sub>2</sub>, and 11 glucose. Upon arrival in the laboratory, the specimen was immediately washed three times with ice-cold, carbogen-aerated Krebs buffer.

### 2.2 | Recordings of secretory activity

Experiments were performed in mucosal/submucosal large intestinal preparations as previously described.<sup>10–12</sup> This required careful removal of the muscle layers with fine forceps and scissors under microscopic control. The final preparations contained the epithelial lining and the submucous layer including all three submucous plexi.

To test the effects of the drugs on ion transport in intact human mucosal/submucosal preparations, we used the Ussing chamber-voltage clamp technique (Easy Mount chambers, Physiologic

Instruments). The tissue specimens were mounted into plexiglass Ussing chambers with a recording area of 1 cm<sup>2</sup>. Mucosal and serosal sides were bathed separately in 5 mL Carbogen-bubbled Krebs solution maintained at 37°C. The set-up allows simultaneous measurements of up to eight mucosal/submucosal preparations dissected from one specimen. We recorded the following parameters that reflected epithelial functions. The short circuit current ( $I_{sc}$ ) is a measure for the transepithelial electrogenic transport (expressed in  $\mu\text{A}/\text{cm}^2$ ; the values were corrected for bath resistance).

Before starting the actual measurements, human tissues were allowed to equilibrate for at least 45 minutes. The drugs were applied basolaterally to the serosal bathing solution.

### 2.3 | Recordings of muscle activity

Transferred specimens were washed three times with ice-cold, carbogenated Krebs buffer. The mucosal and submucosal layers were gently removed with fine forceps and scissors under microscopic control. The methods have been described in detail previously.<sup>10,12,13</sup> Briefly, the preparations were cut parallel to the circular axis to record circular muscle activity. The final size was 1 cm in length and 0.5 cm in width. They were mounted in 15 mL organ baths filled with Carbogen-bubbled Krebs solution and maintained at 37°C by a heated water jacket. After the tension was set to 40 mN, the muscle strips were allowed to equilibrate for at least 60 minutes with renewing the Krebs buffer every 20 minutes till the preparations reached a stable muscle tone. Platinum electrodes were used for transmural electrical field stimulation (EFS) through a constant voltage stimulator (Hugo Sachs, March-Hugstetten, Germany) using 20 V pulses at 10 Hz for 10 seconds with individual pulse duration of 0.5 ms. Drugs were applied to the buffer medium at various concentrations. Their effects on tonic and phasic muscle activities were assessed by analysis of changes in basal muscle tone and motility index. The motility index is a parameter that considers changes in amplitude and frequency of contractions. It was calculated as the area under the curve reflecting changes in amplitude and frequency of contractions. We compared the motility index within a 5 minutes period before addition of peppermint or caraway oil with that in a 5 minutes period when the drugs exerted their maximum effect. In addition, the effects of the drugs on nerve-mediated muscle responses were analyzed.

### 2.4 | Drugs used

Peppermint oil (WS 1340) and caraway oil (WS 1520) were provided by Schwabe (Dr Willmar Schwabe GmbH & Co.KG). Tetrodotoxin (Alomone laboratories) was diluted in deionized water. Bay K8644 (Tocris Bio-Techne) was prepared as a 10 mmol/L stock solution in 100% ethanol. At the final concentrations used, none of the solvents had any effect on muscle or epithelial activity.<sup>12</sup> Addition of the drugs did not change pH or osmolarity of the Krebs buffer.

## 2.5 | Data analysis and statistics

Statistical analyses were performed with Sigmaplot 12.5 (Systat Software Inc). Values are given as median with 25th and 75th quartiles (in the text given in parenthesis separated by/) if not normally distributed or mean  $\pm$  SEM if normally distributed. Concentration-response curves were constructed in a cumulative manner after we verified that the responses to single concentrations did not desensitize. The concentration-response curves were analyzed by Igor Pro 8.0 (Wavemetrics). Coefficient values including the base and max values, Hillslope and  $IC_{50}$  were calculated by fitting concentration-dependent effects with the Hill equation to generate concentration-response curves (Igor Pro 8.0, Wavemetrics). Since  $R^2$  is valid for only linear models, we calculated the standard error of regression, ( $S$ ) which is the appropriate measure for non-linear models (for further details and discussion see <https://statisticsbyjim.com/regression/r-squared-invalid-non-linear-regression/>).  $S$  provides the absolute measure of the typical distance that the data points ( $Y$ ) fall from the regression line ( $Y'$ ) and is a descriptive measure of the accuracy of prediction. It has a unit, in our case either mN for muscle tone or percentage for motility index. The smaller the values the better the fit. The standard error of regression was calculated with the equation  $S = \sqrt{\sum \frac{(Y-Y')^2}{N-2}}$  (<http://onlinestatbook.com/2/regression/accuracy.html>).

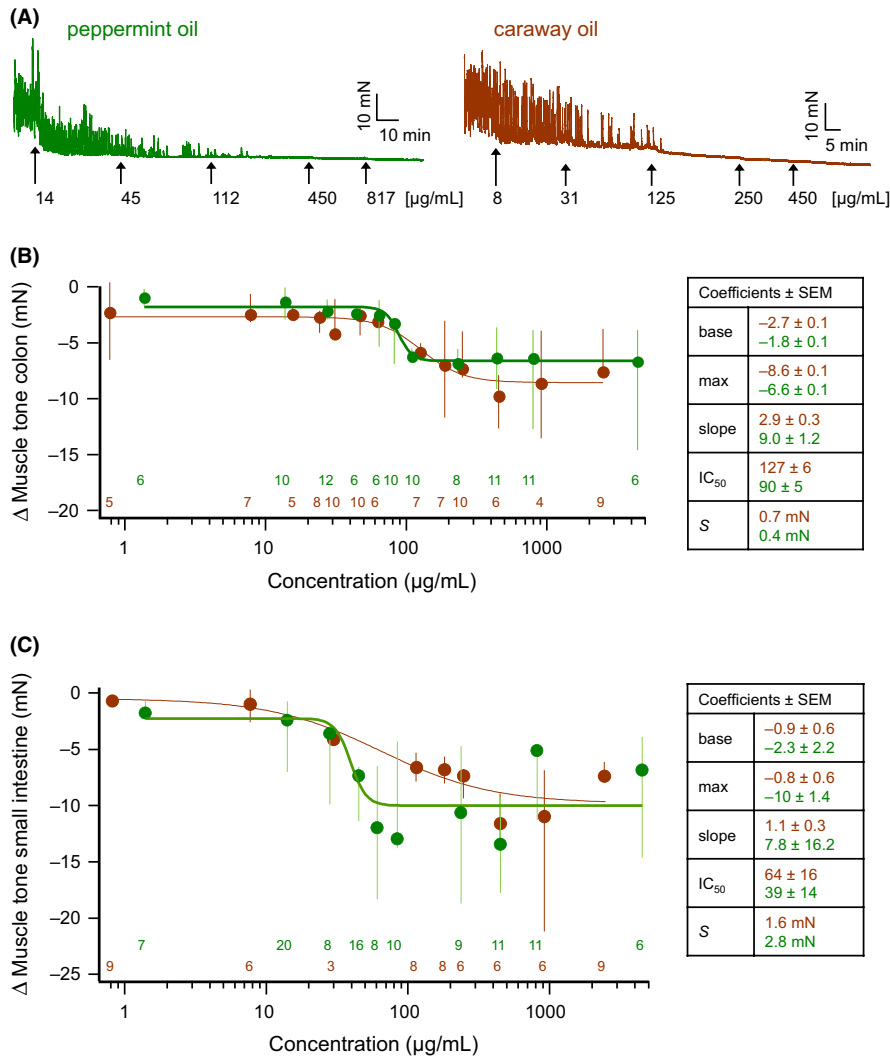
Depending on the data, values were tested with Student's paired or unpaired  $t$  test, one-way repeated measurements ANOVA followed by Holm-Sidak post hoc test, Wilcoxon Signed Rank Test or Friedman Repeated Measures Analysis of Variance on Ranks. Differences were considered significant at  $P < .05$ . The experimental design excluded multiple experiments in tissues from one patient. Therefore, N-number always refers to number of patients.

## 3 | RESULTS

### 3.1 | Motility recordings in muscle strips

Initially, we tested the response to different concentrations (non-cumulative) of peppermint and caraway oils on muscle contractility. We found a reproducible muscle inhibitory effect consisting of a reduction in the amplitude and frequency of phasic contractions and/or a decrease in basal muscle tone. Both effects were slowly developing. With increasing concentrations applied in different tissues, the phasic contractions declined progressively until they ceased. Likewise, the decrease in muscle tone was concentration dependent. Addition of 818  $\mu\text{g}/\text{mL}$  peppermint oil evoked a decline in muscle tone that peaked on average after 13 minutes (range 11-20 minutes). The maximal decrease in tone after 455  $\mu\text{g}/\text{mL}$  caraway oil was reached 11 minutes after addition (range 10-26 minutes). For both drugs, there was no sign of desensitization as the effects were sustained for up to 3 hours, which was the longest recording time.

Cumulative additions of increasing peppermint or caraway oil concentrations evoked increased responses with each increase in



**FIGURE 1** Peppermint and caraway oil concentrations dependently decreased motility in small intestine and large intestine. A, The representative traces illustrate the responses to cumulative application (arrows) of increasing concentrations in two colonic preparations from different patients. Both oils reduce phasic contractions as well as a muscle tone. The concentration-response curves for the decrease in muscle tone in the colon and small intestine are shown in (B) and (C), respectively. Data are median with 25th-75th quartiles. The tables to the right contain the coefficient values, for details see methods. The values above the X-axis correspond to N numbers

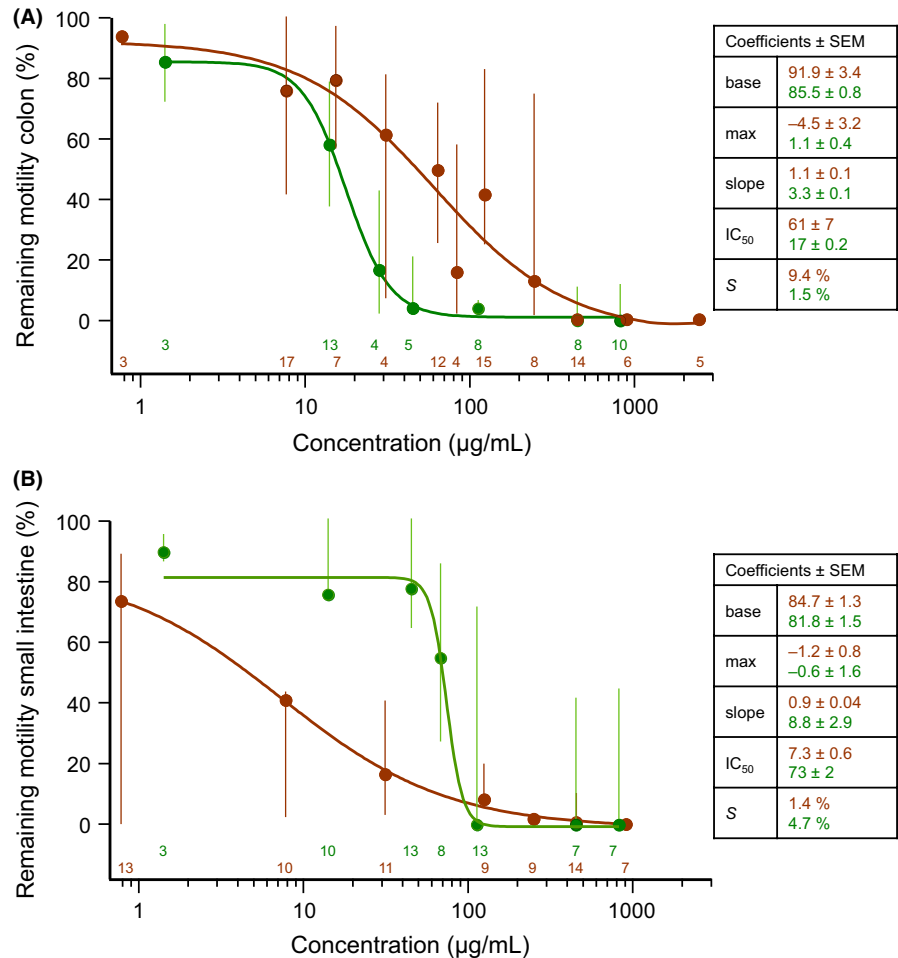
concentration (for example see Figure 1A). Based on the following results, we concluded that the responses to peppermint and caraway oils were independent of whether they were applied cumulatively or non-cumulatively. We first performed non-paired experiments and compared in tissues from different patients the responses to the highest concentrations after cumulative addition or after immediate application. Direct application of 455  $\mu\text{g}/\text{mL}$  caraway oil decreased muscle tone by  $6.3 \pm 0.4 \text{ mN}$  ( $N = 6$ , two small intestine and four large intestine), which was comparable to the decrease observed after cumulative application of increasing caraway applications ( $5.2 \pm 1.2 \text{ mN}$ ;  $N = 5$ , one small intestine and four large intestine;  $P = .66$ ). Direct application of 818  $\mu\text{g}/\text{mL}$  peppermint decreased muscle tone by  $5.5 \pm 0.4 \text{ mN}$  ( $N = 5$ , one small intestine and four large intestine), which was not different from the decrease observed after cumulative application ( $7.4 \pm 1.2 \text{ mN}$ ;  $N = 8$ , three small intestine and five large intestine;  $P = .5$ ). This finding was supported by paired experiments in tissues from four patients (two small intestine and two large intestine). In one sample, we performed the cumulative concentration-response curve, and in the second sample from the same patient, we added only the highest concentration used at the end of

the cumulative addition. The time of application of the highest concentrations in the non-cumulative and cumulative protocol exactly matched to avoid influence of tissue responsiveness with time. The decrease in muscle tone was  $5.2$  ( $12.1/3.9$ )  $\text{mN}$  vs  $4.2$  ( $11.8/3.6$ )  $\text{mN}$  for non-cumulative vs cumulative addition of 455  $\mu\text{g}/\text{mL}$  caraway oil ( $N = 4$  each,  $P = 1.0$ ), respectively. For 818  $\mu\text{g}/\text{mL}$  peppermint oil, the values were  $5.2$  ( $24.8/3.9$ )  $\text{mN}$  vs  $4.6$  ( $11.8/3.8$ )  $\text{mN}$  ( $N = 4$  each,  $P = .6$ ) for non-cumulative vs cumulative application, respectively. Therefore, it was justified to pool the data from experiments using cumulative or non-cumulative concentrations.

The inhibition of phasic contractile activity and the muscle relaxant response was comparable between samples from small or large intestine (compare panels B and C of Figure 1 and panels A and B from Figure 2). The concentration-response curves were generated with different ranges in concentrations as the response strength differed depending on the drugs (peppermint vs caraway oil) and the regions (large vs small intestine).

We analyzed changes in muscle tone (Figure 1) and motility index (Figure 2) in small and large intestinal samples in response to peppermint or caraway oil. The delta change in muscle tone was expressed in

**FIGURE 2** Peppermint and caraway oil concentrations dependently decreased phasic contractility in the human colon (A) and small intestine (B). Data are median with 25th-75th quartiles. The tables to the right contain the coefficient values, for details see methods. The values above the X-axis correspond to N numbers



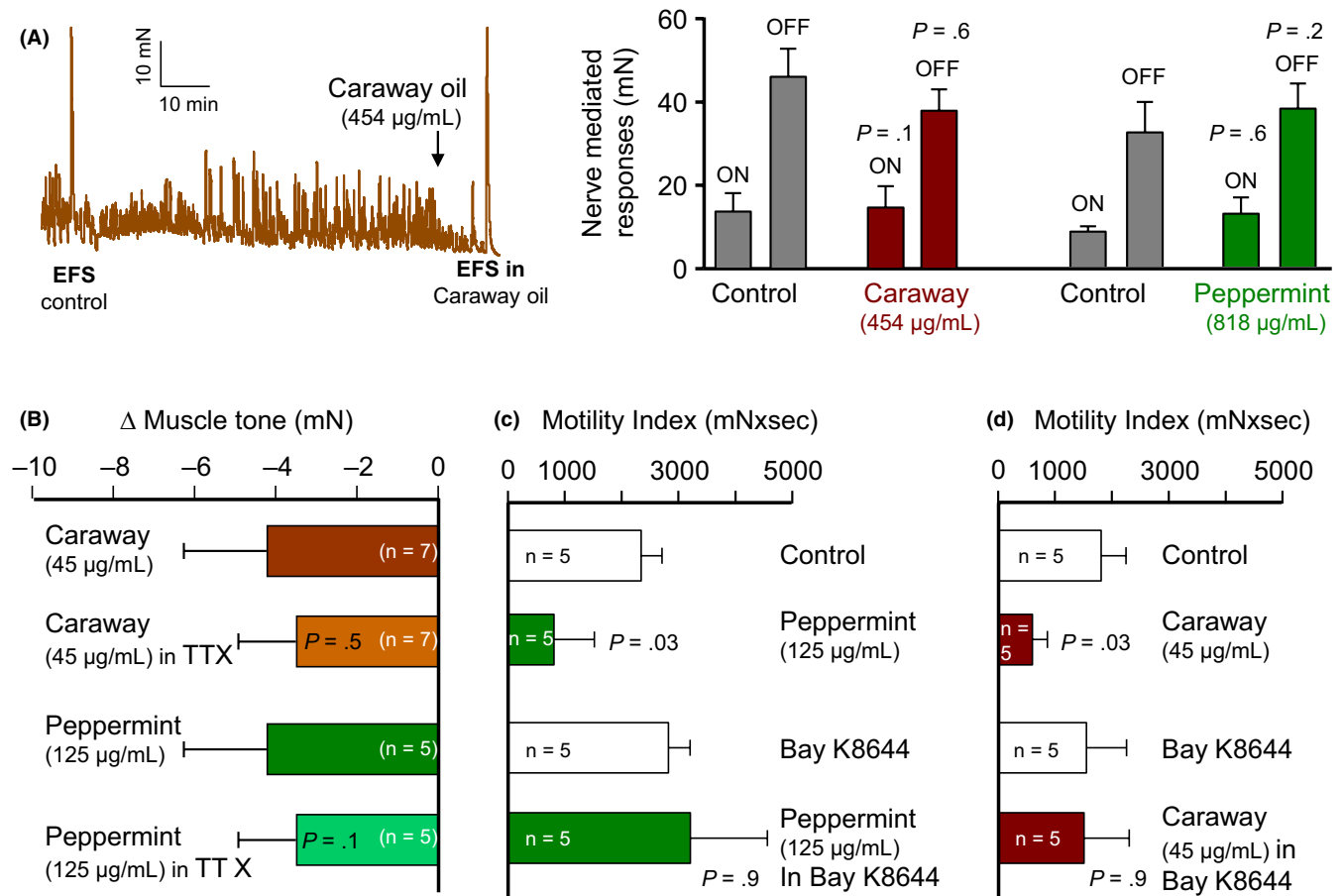
absolute mN values. For the concentration-response curves, the motility index required some additional calculation. This was necessary because, even when present, the contractile activity varied strongly. In order to compensate for the variations during the predrug period, we set the motility index before drug addition to 100% and expressed the reduction as percent remaining motility. In other words, a reduction of the motility index by 30% meant a remaining motility of 70%. Thus, the value 0% indicated that phasic activity completely ceased while a value of 100% indicated no change in motility index.

Cumulative addition of peppermint or caraway resulted in a stepwise decrease in muscle tone as well as in contraction amplitude and frequency (Figures 1 and 2). These effects were seen in the large intestine as well as in the small intestine, and both the decrease in phasic motility and the decrease in muscle tone were comparable between the two regions. In many preparations, even the smallest concentrations caused a reduction in motility. There was no obvious preference toward a decrease in phasic activity or muscle tone as the reductions most frequently started at the same concentration. The IC<sub>50</sub> values were quite comparable suggesting that the efficiency of the drugs was not region-dependent and they had no preferential effects on muscle tone (relaxation) or phasic contractility (Figures 1 and 2).

The results so far showed that peppermint and caraway oils both inhibited motility when applied individually. The medication

Menthacarin<sup>®</sup>, however, contains a combination of caraway and peppermint oils at a ratio of 1:1.8. To test whether peppermint and caraway oils have additive effects, we analyzed changes in muscle tone because the basal phasic activity was too variable (see above). We kept the ratios and used 14.1 µg/mL peppermint oil and 7.8 µg/mL caraway oil as these were the lowest concentrations, which reliably showed a detectable decrease in muscle tone. In addition, we tested six times higher concentrations. Combined application revealed additive but no synergistic effects. Peppermint oil at 14.1 µg/mL evoked a median decrease in muscle tone of 3.1 mN (0.6/10.2) (N = 5 colon) while 7.8 µg/mL caraway oil produced a decline of 0.6 mN (0.1/2.7 mN) (N = 5 colon). Combined application resulted in decrease of the muscle tone of 4.3 mN (3-8.9 mN) (N = 11 colon). This was a clear additive effect, which was not that obvious at the higher concentrations although the combined effect was still greater than the one after individual application of each oil. While the individual application of 84.4 µg/mL peppermint oil and 46.9 µg/mL caraway oil caused a muscle tone decrease of 5.7 mN (2.5/10) (N = 5 colon) and 6.0 mN (0.5/15) (N = 5 colon), respectively, the combined application evoked a decrease of 8.3 mN (4.5/11) (N = 12 colon).

Strikingly, the response to EFS remained unchanged after peppermint or caraway oil (Figure 3). This was not necessarily expected as such strong muscle inhibitory effects might also inhibit



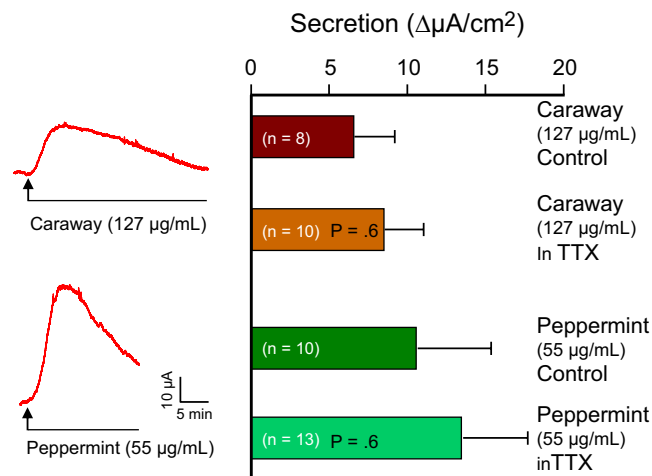
**FIGURE 3** Effects of peppermint or caraway oil did not involve nerves but L-type calcium channels. A, Both oils had no effect on nerve-mediated contractions evoked by EFS. An example trace illustrates the EFS responses (large contractions) before and after application of caraway. Caraway or peppermint oil had no significant influence on the on- or off-responses (compare gray bars with colored bars; P values above the columns). Data are means  $\pm$  SEM (eight small intestine and nine large intestine for caraway; six small intestine and 10 large intestine for peppermint). B, Shows the decrease in muscle tone by caraway (four small intestine and three large intestine) and peppermint (three small intestine and two large intestine) in tissues pretreated with 1  $\mu$ mol/L Tetrodotoxin (TTX) vs non-treated tissues. There is no TTX effect on muscle relaxation. C and D, Show that the L-type Calcium agonist Bay K8644 (1  $\mu$ mol/L) prevented the decrease in phasic contractile activity (Motility index), which was induced by peppermint (C, two small and three large intestine) or caraway oil (D, two small intestine and three large intestine) in control tissues. While peppermint and caraway significantly reduced the motility index in naive tissue, Bay K8644 blocked the inhibitory effects. Data are means  $\pm$  SEM

nerve-mediated responses. We applied high concentrations of the drugs way above the  $IC_{50}$  values to safely conclude about influences on nerve-mediated responses. The EFS-evoked contractile responses that consisted of two components. The first one occurred during the nerve stimulation, hence the term on-response. The second is the off-response as this contraction started only after termination of the electrical stimulus. Neither caraway nor peppermint oil had any significant effects on the on- or the off-response (Figure 3A).

The lack of effect on the nerve-mediated contractile response already suggested that the effects of the two oils were likely nerve-independent. Nevertheless, we addressed this point by comparing the effects of peppermint and caraway oils in naive tissue vs tissues pretreated with the nerve blocker tetrodotoxin (1  $\mu$ mol/L, Figure 3B). The muscle relaxant effects of peppermint and caraway oils remained in tetrodotoxin treated tissues. The parameter analyzed was muscle tone as phasic contractions under basal conditions were too variable (see above).

This result leaves a myogenic mechanism as one likely target for peppermint and caraway oils. Based on the strong inhibitory responses, we hypothesized an inhibition of L-type calcium channels as one mode of action. To overcome this inhibition, we used the high affinity activator of L-type calcium channels Bay K8644 (1  $\mu$ mol/L). Bay K8644 did activate phasic contractions in all tissues also in those, which stayed quiescent throughout the predrug period. In addition, Bay K8644 raised the muscle tone. We analyzed the area under the curve, which would represent the frequency and amplitude of contractions. In control tissues exhibiting spontaneous contractions peppermint and caraway inhibited the contractile activity and hence reduced the motility index. Bay K8644 prevented this inhibitory action (Figure 3C and D). Unexpectedly, Bay K8644 increased the peppermint and caraway induced muscle relaxation when comparing the effects of the oils in control tissue with Bay K8644 pretreated tissues from the same patient. In control preparations, 125  $\mu$ g/mL caraway oil reduced the muscle tone by  $4.2 \pm 1.4$  mN whereas the same





**FIGURE 4** Peppermint and caraway oils increased anion secretion in the human colon. The left panel shows representative traces illustrating the increase in short circuit current after serosal addition of peppermint oil or caraway oil. The panel on the right shows the response amplitude and the finding that the nerve blocker tetrodotoxin (TTX) did not affect the response. Data expressed as means  $\pm$  SEM

concentration induced a larger (non-significant) relaxation in Bay K 8644 pretreated tissues from the same patients ( $17.5 \pm 5.2$  mN, three small intestine and five large intestine,  $P = .144$ ). While  $45 \mu\text{g/mL}$  peppermint oil decreased muscle tone by  $1.9 \pm 1.6$  mN, the same concentration evoked a much larger relaxation in Bay K 8644 pretreated tissues from the same patients ( $14.8 \pm 4.6$  mN, three small intestine and six large intestine,  $P = .018$ ).

### 3.2 | Effects of peppermint oil and caraway oil on epithelial secretion

The studies on epithelial secretion were performed in colonic preparations and primarily intended to discover possible effects of  $127 \mu\text{g/mL}$  caraway oil or  $55 \mu\text{g/mL}$  peppermint oil on epithelial secretion (Figure 4). At these concentrations, we detected muscle relaxations. The drugs were applied onto the serosal side of the preparation, which mimicked the effects once they were absorbed. Shortly after application the baseline started to increase and reached a maximum within 5-10 minutes. Then, the response declined slowly until it reached the predrug level again. Unlike the effects on motility, the pro-secretory actions were not sustained despite continuous presence of the drugs. The effect was tetrodotoxin resistant indicating a direct activation of epithelial cells. The response amplitude after peppermint or caraway oil is comparable to that seen after EFS-induced secretion (this study and<sup>11</sup>).

## 4 | DISCUSSION

This study revealed three main findings. First, peppermint and caraway oils significantly decreased motility in the small intestine and

large intestine by reducing muscle tone and phasic contractility. Second, the inhibition was myogenic involving inhibition of L-type calcium channels. Third, peppermint and caraway oils increased ion secretion into the lumen via direct epithelial activation not involving nerves.

One question that always arises when discussing in vitro effect of drugs is the clinical relevance of the findings and their translation to the in vivo situation. One capsule of Menthacarin<sup>®</sup> contains 90 mg peppermint oil and 50 mg caraway oil. The recommended daily dose is two capsules distributed during the day. The plasma concentrations of menthol and carvone, two of the most prominent constituents in peppermint and caraway oils, after application of two capsules to healthy volunteers were around 1000 ng/mL for menthol and around 10 ng/mL for carvone.<sup>14</sup> Although it is difficult to relate the in vitro concentrations applied to our tissue preparations to effective doses in humans, we may at least discuss some relevant issues. From the outset, there are at least two important considerations that need to be discussed. First, it is most likely that the concentrations in the gut wall are much higher than those measured in the plasma (for discussion see<sup>10,12</sup>). Second, the usually assumed intestinal volumes are vastly overestimated. Thus, the volume in the small intestine and large intestine was 120 mL after a 250 mL test drink,<sup>15</sup> and the postprandial volume in the colon was around 500 mL.<sup>16</sup> Essential oils are well absorbed, and it is generally assumed that menthol is completely absorbed in the gut.<sup>5</sup> This is most likely also the case for carvone but has so far not been measured. Considering the above, the luminal concentration of the constituents of peppermint and caraway oils after taking one capsule may add up to 180-750  $\mu\text{g/mL}$  and 100-415  $\mu\text{g/mL}$ , respectively. This is in the range used in our study.

The inhibitory effect on phasic muscle contractility but not the decrease in muscle tone was prevented by the L-type calcium channel activator Bay K8644. It seems unlikely that peppermint oil and caraway oil displace Bay K8644 from its binding site and therefore may overcome the Bay K 8644 agonistic actions on L-type calcium channels. If that would be the case, Bay K8644 would not have prevented the action on phasic contractile activity. Of course, we cannot exclude that higher concentrations of Bay K8644 would block the effect of peppermint oil and caraway oil on muscle tone. Alternatively, peppermint and caraway may have additional effects on other calcium dependent mechanisms. It has been shown in *Helix* neurons that menthol blocks L- and T-type calcium channels.<sup>17</sup> T-type calcium channels are expressed on human colonic smooth muscles and some spasmolytic-acting drugs seem to act as blockers of L- and T-type calcium channels.<sup>18</sup> An effect of menthol and peppermint oil on non-L-type calcium channels was proposed for gastrointestinal and cardiac muscle as well as for retinal synapses.<sup>19</sup> TRPM8 channels are unlikely to be involved in peppermint actions on intestinal smooth muscle because menthol activates calcium influx through this channel.<sup>20</sup> Menthol inhibited store operated calcium influx in platelets.<sup>21</sup> Although intracellular calcium stores play a relatively minor role in intestinal contractility, their blockade causes muscle relaxation

by inhibition of intracellular calcium release or store operated calcium entry in interstitial cells of Cajal<sup>22</sup> and smooth muscle cells.<sup>23</sup> Additionally, the strong increase in muscle tone by Bay K8644 renders such precontracted preparation more sensitive to relaxation than preparations with less tone.

At low concentrations, we have seen an additive effect of peppermint and caraway oils that was not present at the 6× higher concentrations. This may be related to common targets for the two oils, which at some point involve L-type-calcium channels. It is plausible that a strong inhibition by one oil limits the inhibition by the other oil.

All in all, our findings are supported by previous studies although an inhibition on human intestinal muscle by peppermint oil and caraway oil has not been directly demonstrated before. Menthol relaxed human colon preparations, an effect mediated by L-type calcium channels.<sup>4</sup> Likewise, carvone and limonene, two constituents of caraway oil, inhibited contractions in guinea pig ileum preparations.<sup>24</sup> Based on similar effects of the calcium channel blocker verapamil, it was suggested that the antispasmodic effects of carvone were mediated by a similar action.<sup>25</sup> In another study, peppermint oil markedly attenuated basal and agonists-induced contractions in guinea and rabbit intestinal muscle strips.<sup>26</sup> Based on patch clamp recordings, the authors concluded that this inhibition was due to reduced calcium influx.

One study on human colon muscle strips reported that 1-3 mmol/L menthol strongly reduced EFS-evoked atropine-sensitive contractions and the carbachol-contractile responses.<sup>4</sup> This high menthol concentration was cytotoxic in other cell models.<sup>27</sup> In our experiments, we estimate the menthol concentration to be in the low  $\mu$ M range (0.26  $\mu$ mol/L at 100  $\mu$ g/mL peppermint oil).

Likewise, ethanolic extracts of *Carum carvi* reduced the response to acetylcholine in guinea pig dispersed intestinal smooth muscle cells.<sup>28</sup> Carvacrol a constituent of caraway oil relaxed rat aorta by blocking calcium influx and by interfering with intracellular calcium handling.<sup>29</sup>

The inhibition of motility by peppermint and caraway oils did not involve nerves because it was not sensitive to tetrodotoxin. Reduced efficacy of acetylcholine was not the reason for muscle inhibition in our study, as the EFS-evoked contractile on-response, which is in human intestinal preparations abolished by cholinergic muscarinic receptor blockade,<sup>10</sup> was not influenced. This is puzzling at first sight, as one would expect that a strong inhibition of phasic and tonic muscle activity would attenuate cholinergic contractions, in particular since activation of muscarinic receptors would require opening of calcium channels to evoke contractions. However, the inhibition of acetylcholine evoked responses in guinea pig ileum by calcium channel blockers is rather moderate.<sup>30</sup> Even 10  $\mu$ mol/L verapamil reduced acetylcholine evoked phasic contractions only by 60%; the same was true for the amplitude of the twitch response. The cholinergic mediated excitatory junction potentials were only reduced by about 30% in 10  $\mu$ mol/L nifedipine. This is also supported by our own unpublished data in human intestinal samples: 1  $\mu$ mol/L nifedipine had no effect on electrical field stimulation evoked responses but it required 10  $\mu$ mol/L to reduce the response to about 50%.

It seems that the supramaximal field stimulation releases enough acetylcholine to overcome inhibition of L-type calcium channels by peppermint or caraway oil. This may be due to displacement, compensatory mechanisms linked to intracellular calcium stores or Ca-entry through non-L-type channels.<sup>30</sup>

The finding that the nerve-mediated response was not significantly affected by the two oils suggested that neither peppermint nor caraway paralyzed the gut but their effects were more modulatory. This is particularly relevant for the in vivo situation because proper function of the gut requires that the neurally mediated peristaltic reflex is still operative.<sup>31,32</sup>

Our findings showed a pro-secretory action of peppermint and caraway oils, which contrasts the inhibitory effect of peppermint oil on acetylcholine induced secretion in rat small intestine.<sup>33</sup> The discrepant results may be species-related but more likely are due to the high concentration (1-5 mg/mL) used in the rat study. There are no data regarding effects of caraway oil on mucosal secretion besides the finding that caraway fruits extract had no influence.<sup>9</sup> In the human colon, the increase in short circuit current is mediated by secretion of anions into the lumen and carried by apical chloride and bicarbonate fluxes.<sup>11</sup> Although we did not perform ion substitution experiments, we therefore conclude that peppermint and caraway evoked increases in short circuit current was mediated by luminal secretion of chloride and bicarbonate ions.

It seems odd for a drug to inhibit muscle activity but at the same time to activate epithelial secretion. However, this is exactly how ENS transmitters act under physiologic conditions. Vasoactive intestinal polypeptide and nitric oxide are two prominent examples of transmitters, which inhibit muscle activity but stimulate secretion.<sup>11</sup> It seems that the gut uses these "opposing" actions to keep the content moist even in a quiescent intestine. That may be a protective mechanism in order to keep the luminal bolus soft enough to be transported even with shallow contractions.

In conclusion, our study revealed a strong inhibition of motility and a pro-secretory action of peppermint and caraway oils at clinically relevant concentrations. Both actions were nerve-independent. Neither peppermint nor caraway oil paralyzed the gut but their effect was modulatory. The inhibition of contractility was mediated by inhibition of L-type calcium channels. The exact mechanisms responsible for the strong inhibitory influence on muscle tone remain open. We conclude that the effects on muscle and epithelial activity may contribute to the beneficial effects observed in patients with FGD.

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## CONFLICT OF INTEREST

None.



## AUTHOR CONTRIBUTIONS

DK performed and analyzed experiments; SS performed Ussing chamber experiments; FZ, IED, and JT provided tissues, KM provided statistical analysis, MS designed the study and wrote manuscript. All authors approved manuscript.

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