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Coumarin Inhibits the Contractile Activity of the Duodenal Visceral Smooth Muscle by NO Mediated–cGMP Signalling Pathway

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ABSTRACT

Coumarin, a naturally occurring aromatic compound found in many plants, has been widely studied for its pharmacological properties. However, its effects on the contractions of the visceral smooth muscles located in the wall structure of the small intestine remain underexplored. Therefore, this study aimed to examine the effects of coumarin on the contractions of the duodenal visceral smooth muscle (dVSM) in rats. through *ex vivo* recordings of the movement of duodenum, a representative and initial part of the small intestine, using an isotonic transducer (IT-2245) connected to RMS Polyrite D. Coumarin induced a significant, concentration-dependent suppression of duodenal contractions, as evidenced by decrease in amplitude and frequency of spontaneous contractions. From this result, it could be hypothesised that the Coumarin induced inhibition of the contractions of the dVSM might be due to suppression of the activity of intrinsic cholinergic myenteric neurons and/or facilitation of the activity of intrinsic adrenergic/ nitrergic myenteric neurons. Further, to understand the probable pharmacodynamic mechanisms involved in the Coumarin induced inhibition of the contractions of the dVSM, we focused on the involvement of nitrergic nitric oxide (NO)-cGMP signalling pathway, a key regulatory mechanism involved in smooth muscle relaxation. From the single dose experiments, it has been observed that pre-treatment with L-NAME and methylene blue (MB) further significantly counteracted the inhibitory effect of coumarin on the dVSM contraction, suggesting that coumarin may stimulate endogenous NO production or interfere with upstream cGMP signalling. In contrast, administration of sodium nitroprusside significantly suppressed both spontaneous and coumarin-induced contractions, confirming the functional integrity of the nitrergic relaxation pathway and its agonism by coumarin. The findings indicate that coumarin decreases the duodenal visceral smooth muscle contraction by facilitating the nitrergic NO–cGMP signalling pathway. These results provide novel insights into the action of coumarin on SiVSM upon dietary exposure.

Keywords: Coumarin, duodenal visceral smooth muscle, intrinsic myenteric neurons,

nitrergic signalling pathway, Nitric Oxide (NO)-cGMP signalling pathway

INTRODUCTION

Coumarin (1-benzopyran-2-one), a naturally occurring phytochemical found in *tonka beans* (*Dipteryx odorata*), *sweet clover* (*Melilotus officinalis*), and *cassia cinnamon* (*Cinnamomum cassia*), is known for its diverse pharmacological effects, including anti-inflammatory, antimicrobial, antioxidant, and anticoagulant properties (Venugopala et al., 2013; Lake, 1999; Borges et al., 2005). Structurally, Coumarin serves as the parent compound for several clinically important drugs, such as warfarin and other vitamin K antagonists (Abraham et al., 2010). There is increasing concern regarding the physiological consequences of excessive Coumarin intake, particularly through dietary supplements or as an adulterant in food. Although the compound is banned as a food additive in several countries due to its hepatotoxic potential, natural exposure remains common through herbal products and spices (Abraham et al., 2010; Lake, 1999). Today, many commercially available goods are spiced with Cassia cinnamon, and hence contain coumarin. In general, cinnamon sticks and ground cinnamon have varying levels of coumarin; cinnamon sticks often contain more coumarin than ground cinnamon (Ballin and Sørensen, 2014). As a result, the presence of coumarin in cinnamon has raised concerns, as coumarin levels in some goods can exceed the limitations imposed by European legislation (Wang et al., 2013). According to Italian research, over 70% of the cinnamon-flavored goods tested contained more coumarin than authorized (Lungarini et al., 2018).

The contractile activity of the small intestine is governed by the coordinated activity of smooth muscle contractions, regulated by the enteric nervous system (ENS) through a balance of excitatory and inhibitory neurotransmitters (Wood, 1999). The contractions of the SiVSM provides motility to the small intestine that aids to perform its digestive and absorptive functions. Any exposure to environmental agents might exert effects on the contractions of the SiVSM that led to impaired digestive and absorptive functions.

Earlier research has shown that synthetic dyes and environmental toxins can interfere with GI motility by modulating nitrergic or cholinergic pathways (Ghosh et al., 2021). These findings raise the question of whether Coumarin, despite being a natural compound, might similarly alter neuromuscular transmission in the small intestine. So, to examine the potential adverse effects of Coumarin on the motor function of the SiVSM, the present study focused on the contractions of the duodenal visceral smooth muscle (dVSM)—a key component of the small intestine involved in regulating motility.

MATERIALS & METHODS

Chemicals and Reagents

All the reagents and chemicals that were used to conduct this study were of analytical grade. The test chemical- Coumarin (≥98% purity) was procured from Sigma-Aldrich. N-ω-nitro-L- arginine methyl ester (L-NAME) hydrochloride has been purchased from Sigma Aldrich, USA. Methylene blue (MB) and sodium nitroprusside (SNP), sodium chloride (NaCl), potassium chloride (KCl), magnesium chloride ($MgCl_2$), calcium chloride ($CaCl_2$), sodium bicarbonate ($NaHCO_3$), sodium dihydrogen phosphate (NaH_2PO_4), glucose, etc. were procured from E. Merck, India.

Figure1: Chemical structure of coumarin

Experimental Animals

Adult female Sprague Dawley rats (130–150 g, 2–3 months old) were maintained under standard laboratory conditions (12 h light/dark cycle, 25–27°C) with access to

food and water ad libitum. All procedures were conducted in accordance with the guidelines of the Animal Ethics Committee, University of Kalyani.

Experimental Animals and Care

As the experimental model, adult female albino rats of Sprague Dawley strain with body weight ranging around 130-150 g and age around 2-3 months were selected. They were kept in the room temperature of 25-27°C at the departmental animal care room with 24 hours light-dark cycle and were fed with laboratory chow and water and were kept in the animal house in accordance with the animal ethics committee's guidelines from Kalyani University.

Experimental Design

The animals were treated to different exposure conditions as mentioned in Table 1.

Table 1: Experimental Setup for the study

Groups	Exposure condition
Set 1	Application of graded doses of Coumarin (4, 8, 16, 32 μM) on the duodenal segments
Set 2	Application of single dose of SNP (2.5μM) on the duodenal segments
Set 3	Application of effective dose of Coumarin on duodenal segments pretreated with SNP
Set 4	Application of single dose of L-NAME (200 μM) on the duodenal segments
Set 5	Application of effective dose of Coumarin on duodenal segments pretreated with L-NAME
Set 6	Application of single dose of MB (200 μM) on the duodenal segments
Set 7	Application of effective dose of Coumarin on duodenal segments pretreated with MB

Animal Sacrifice

he selected animals were subjected to overnight fasting prior to sacrifice to standardize physiological conditions. Euthanasia was performed using cervical dislocation, ensuring minimal pain and distress, in strict accordance with the ethical guidelines approved by the Animal Ethics Committee of the University of Kalyani.

Collection of the Organ

Following cervical dislocation and confirmation of death, the animal's abdominal cavity was opened. The small intestine was carefully dissected free from the mesentery, stomach, and large intestine via transverse incisions. The proximal portion of the small intestine, specifically the duodenum, was isolated for the study, as it exhibits the most prominent motility among intestinal segments. The collected duodenal segment was immediately transferred to a beaker containing temperature-controlled Tyrode's solution. The lumen was gently flushed to remove any residual contents. The cleaned segment was then promptly mounted in the organ bath of Dale's apparatus for ex vivo recording of spontaneous duodenal motility.

Recording of the Movement of the Duodenum

To record the spontaneous ex vivo motility of duodenal visceral smooth muscle (dVSM), a duodenal segment approximately 3 cm in length was vertically suspended in an organ bath containing 40 ml of Tyrode's solution. The segment was secured using two metal hooks inserted at both ends of the tissue. The composition of Tyrode's solution included: 8.0 g NaCl, 0.2 g KCl, 0.2 g $CaCl_2$, 0.1 g $MgCl_2$, 0.05 g NaH_2PO_4, 1.0 g $NaHCO_3$, and 1.0 g dextrose per litre, adjusted to pH 7.4. Oxygenation was maintained using a continuous flow of oxygen at a rate of 2–3 bubbles per second, delivered directly into the organ bath via an oxygen bubbler. The bath temperature was kept at 37 ± 0.5°C using an automatic thermostat integrated with Dale's apparatus. The lower end of the duodenal tissue was anchored to the base of the organ bath, while the upper end was connected to the lever of an isotonic transducer (IT 2245). The transducer was interfaced with RMS Polyrite-D software (RMS, Chandigarh, India) to enable continuous recording of tissue contractions. Each tissue segment was

allowed to stabilize for at least 35 minutes under these experimental conditions and was rinsed multiple times with fresh Tyrode's solution to remove metabolic residues. Isotonic contractions representing spontaneous rhythmic motility were recorded continuously following the administration of various concentrations of coumarin and selected pharmacological blockers.

STATISTICAL ANALYSIS

Data from each experimental group were expressed as mean ± SEM. The frequency and amplitude of the recorded duodenal movements were analyzed to determine the contractile force. For functional assessments, the responses of treated tissues were calculated as percentage changes relative to their respective basal (control) values. Statistical comparisons among groups were performed using one-way ANOVA with GraphPad Prism 8 software. A *P*-value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Effect of Graded Doses of Coumarin on the Movement of Isolated Duodenum *Ex Vivo* of Rat

To study the effect of Coumarin on the contractile activity of the duodenal visceral smooth muscle (dVSM), *ex vivo* recordings of duodenal movements were obtained from isolated duodenal segments exposed to graded concentrations of Coumarin in single-dose acute experiments. Analysis of the tracings revealed that Coumarin caused a dose-dependent decrease in the amplitude of contractions. Additionally, the frequency of contractions was also progressively reduced with increasing concentrations of Coumarin (Figure 2 and Figure 3). The suppression of duodenal motility remained consistent throughout the 20-minute observation period. Notably, at the 160 µM concentration, Coumarin inhibited duodenal motility by over 80% compared to controls, with contractile activity virtually abolished for the remainder of the experimental duration.

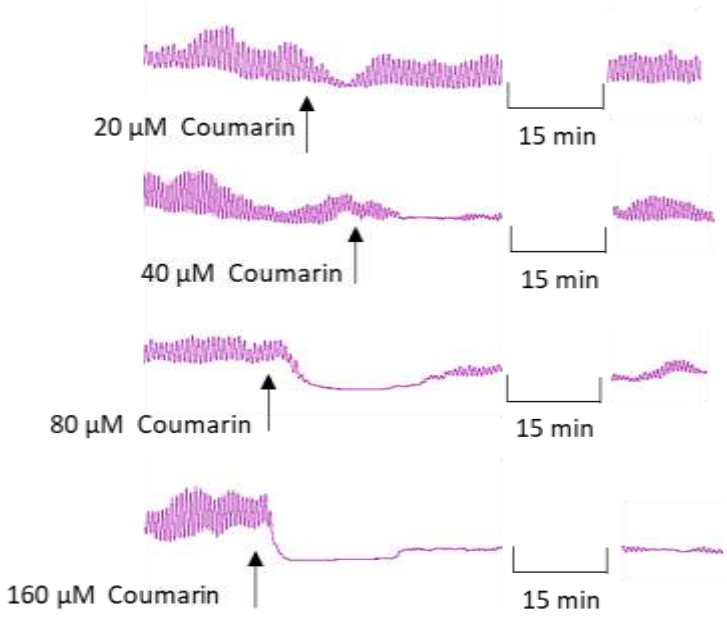

Figure 2. Tracings showing representative records of the effect of graded concentrations of Coumarin on the isolated duodenal segment in order to examine the effect of Coumarin on the contractile activity of the SiVSM in rat *ex vivo* obtained with an isotonic transducer coupled to RMS Polyrite-D.

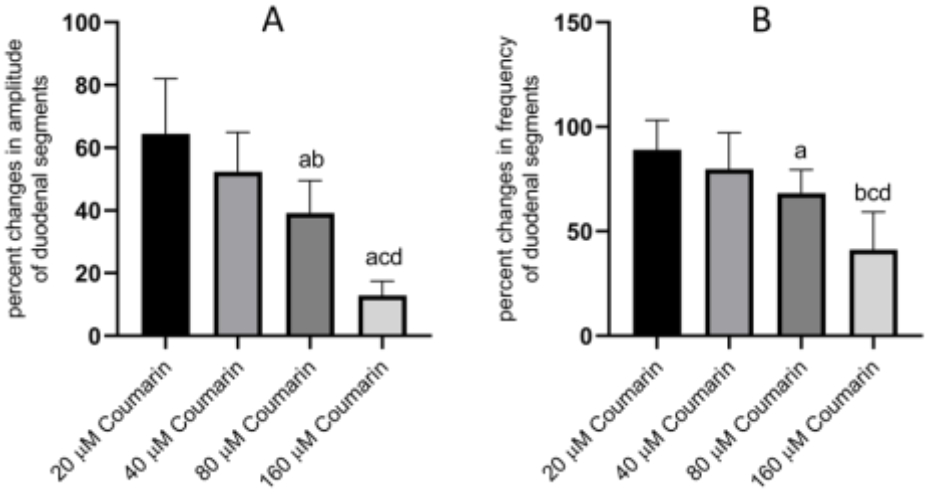

Figure 3. Bar diagrams showing the percent changes in the amplitude (A) and frequency (B) of contraction of the duodenum in Coumarin exposed groups (20 µM, 40 µM, 80 µM, 160 µM) compared to control. The data were represented as mean ± SEM for all the groups. [a]p<0.0001 vs. 20 µM Coumarin, [b,c]p< 0.05, 0.0001 vs. 40 µM Coumarin and [d]p<0.0001, vs. 80 µM Coumarin (A). [a,b]p<0.01,0.0001 vs. 20 µM Coumarin, [c]p<0.0001 vs. 40 µM Coumarin and [d]p<0.01, vs. 80 µM Coumarin (B).

Based on the results, it can be inferred that Coumarin suppresses the contractile activity of the duodenal visceral smooth muscle (dVSM) primarily by reducing the amplitude of contractions. Although a decrease in contraction frequency was initially observed, no statistically significant change in frequency was ultimately examined. It can be suggested that the Coumarin-induced suppression of dVSM contractility may be due to either inhibition of excitatory cholinergic myenteric efferents and/or activation of inhibitory adrenergic and/or nitrergic (NANC) pathways innervating the dVSM. Further, the alteration in the frequency of contractions might be due to modulation in the generation of slow waves that determines the rhythmicity of the contractions of the dVSM.

Effect of Coumarin on the Movement of Isolated Duodenum Pre-Incubated With SNP

To study the potential pharmacodynamic mechanisms underlying in the Coumarin induced inhibition of the contraction of the duodenal visceral smooth muscle (dVSM), the role of nitrergic intrinsic myenteric efferents was examined. These efferents are known to exert an inhibitory effect on small intestinal smooth muscle contraction by releasing nitric oxide (NO), a key inhibitory neurotransmitter that promotes smooth muscle relaxation. To assess the contribution of the nitrergic myenteric efferents in Coumarin -induced suppression of dVSM contractions, *ex vivo* recordings of duodenal motility were conducted in single-dose acute experiments following co-application of Coumarin and Sodium Nitroprusside (SNP), a nitrergic agonist and NO donor. As evidenced from the tracings, the extent of inhibition of dVSM contractions was significantly pronounced in SNP pre-treated preparations compared to Coumarin alone (Figure 4). This synergistic inhibitory effect on the dVSM contractions strongly suggests that Coumarin inhibits the contraction of the dVSM probably by activating nitrergic myenteric efferents, that secrete NO, the smooth muscle relaxant.

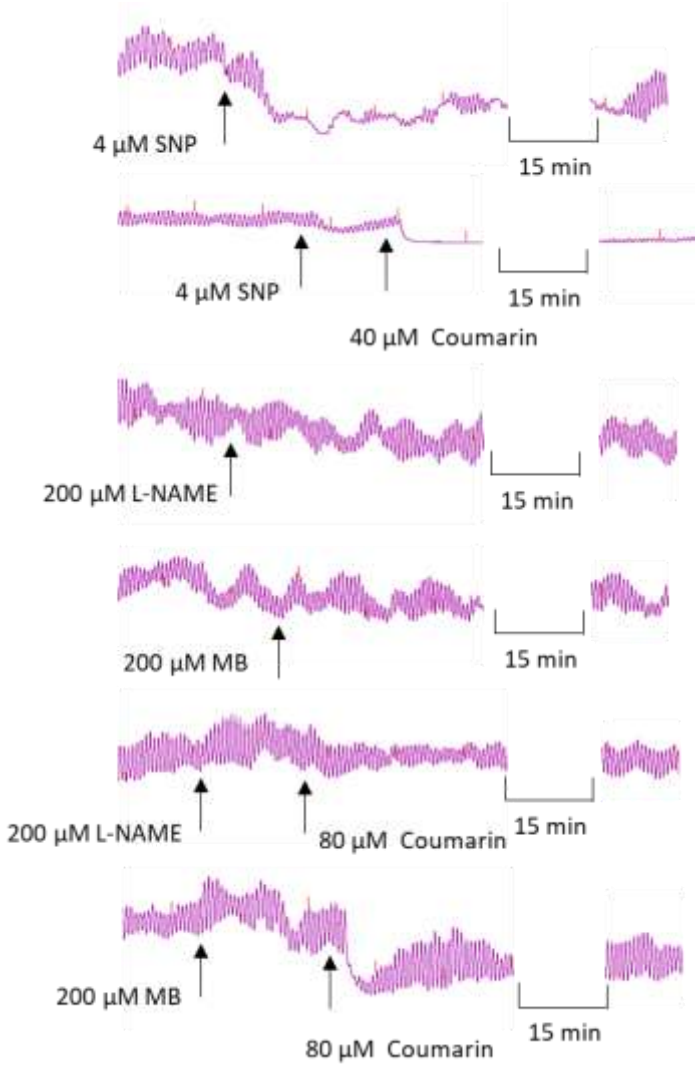

Figure 4. Representative tracings showing the effects of Coumarin in combination with nitrergic agonist and nitrergic antagonist on the movement of duodenum *ex vivo* of rat in single dose acute exposure in order to examine the involvement of nitrergic signalling pathway in Coumarin induced inhibition of the contraction of the duodenal visceral smooth muscle.

Effect of Coumarin on the Movement of Isolated Duodenum Pre-incubated with L-NAME

To assess the involvement of nitrergic myenteric efferents in Coumarin-induced suppression of duodenal visceral smooth muscle (dVSM) contraction, the movement of the duodenum in response to the application of L-NAME (Nitric oxide synthase inhibitor), and co-application of Coumarin and L-NAME, were recorded with the help of an isotonic transducer coupled to RMS Polyrite D. The tracings revealed that L-NAME alone did not produce any significant changes in dVSM contractions. However, when Coumarin was applied in combination with L-NAME, the inhibitory effect of Coumarin on dVSM contractility was notably counteracted compared to the suppression observed with Coumarin alone (Figure 4 and Figure 5). These findings suggest that the Coumarin inhibits the contraction of the dVSM probably by facilitating the activity of nitrergic myenteric efferents through increase in the production of nitric oxide due to augmentation in the activity of nitric oxide synthase as a result of Coumarin induced intoxication in the dVSM.

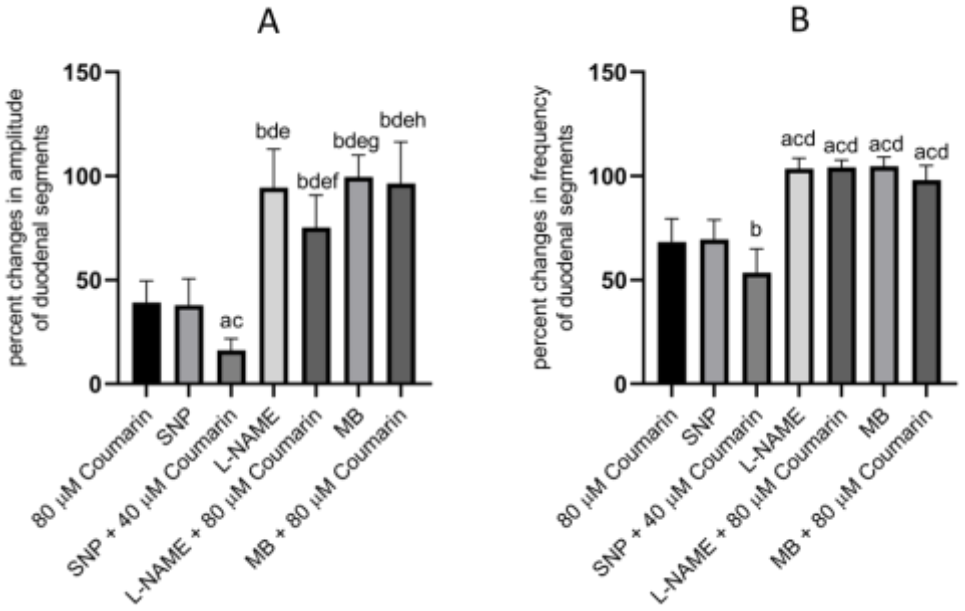

Figure 5. Bar diagrams showing the percent changes in the amplitude (A) and frequency (B) of contraction of the duodenum in response to the application of Coumarin in combination with nitrergic agonist and nitrergic antagonist. The data were represented as mean ± SEM for all the groups. [a,b]p<0.01, 0.0001 vs. 80 µM Coumarin, [c,d]p< 0.01, 0.0001 vs. SNP, [e]p<0.0001 vs. SNP + 40 µM Coumarin, [f]p<0.01 vs. L-NAME, [g,h]p<0.001, 0.01 vs. L-NAME + 80 µM Coumarin (A). [a]p<0.0001 vs. 80 µM Coumarin, [b,c]p< 0.001, 0.0001 vs. SNP, [d]p<0.0001 vs. SNP + 40 µM Coumarin (B).

Effect of Coumarin on the Movement of Isolated Duodenum Pre-Incubated With MB

To evaluate the involvement of the nitrergic signaling pathway in Coumarin-induced suppression of duodenal visceral smooth muscle (dVSM) contractility, *ex vivo* single-dose acute experiments were performed by recording duodenal movements in response to the combined application of Coumarin and Methylene Blue (MB), a nitrergic antagonist that blocks soluble guanylyl cyclase (sGC). The experimental tracings indicated that MB alone did not produce any significant alteration in dVSM contractions. However, when Coumarin was co-administered with MB, the inhibitory effect of Coumarin on dVSM contractions was markedly attenuated compared to the suppression observed with Coumarin alone (Figure 5). These results strongly suggest that Coumarin exerts its inhibitory action via activation of the sGC-mediated nitrergic signalling pathway.

Among the key inhibitory mediators, nitric oxide (NO)—produced by neuronal nitric oxide synthase (nNOS)—plays a central role in promoting smooth muscle relaxation in the GI tract, particularly in the duodenum (Bult et al., 1990). NO activates soluble guanylate cyclase (sGC) in smooth muscle cells, which increases the levels of cyclic guanosine monophosphate (cGMP), leading to inhibition of contractile activity (Murthy, 2006). Disruption of this nitrergic NO–cGMP signalling pathway can tilt the balance toward excitatory neurotransmission, resulting in altered gut motility patterns and increased muscle tone (Bult et al., 1990; Hirano et al., 1997). The results indicates that both nitrergic antagonists, L-NAME and Methylene Blue (MB), effectively counteracted the Coumarin-induced inhibition of dVSM contractions. This strongly suggests that Coumarin suppresses dVSM contractility via a nitric oxide-mediated, soluble guanylyl cyclase (sGC) signaling pathway, likely by enhancing the activity of intrinsic nitrergic myenteric efferents (Figure 6).

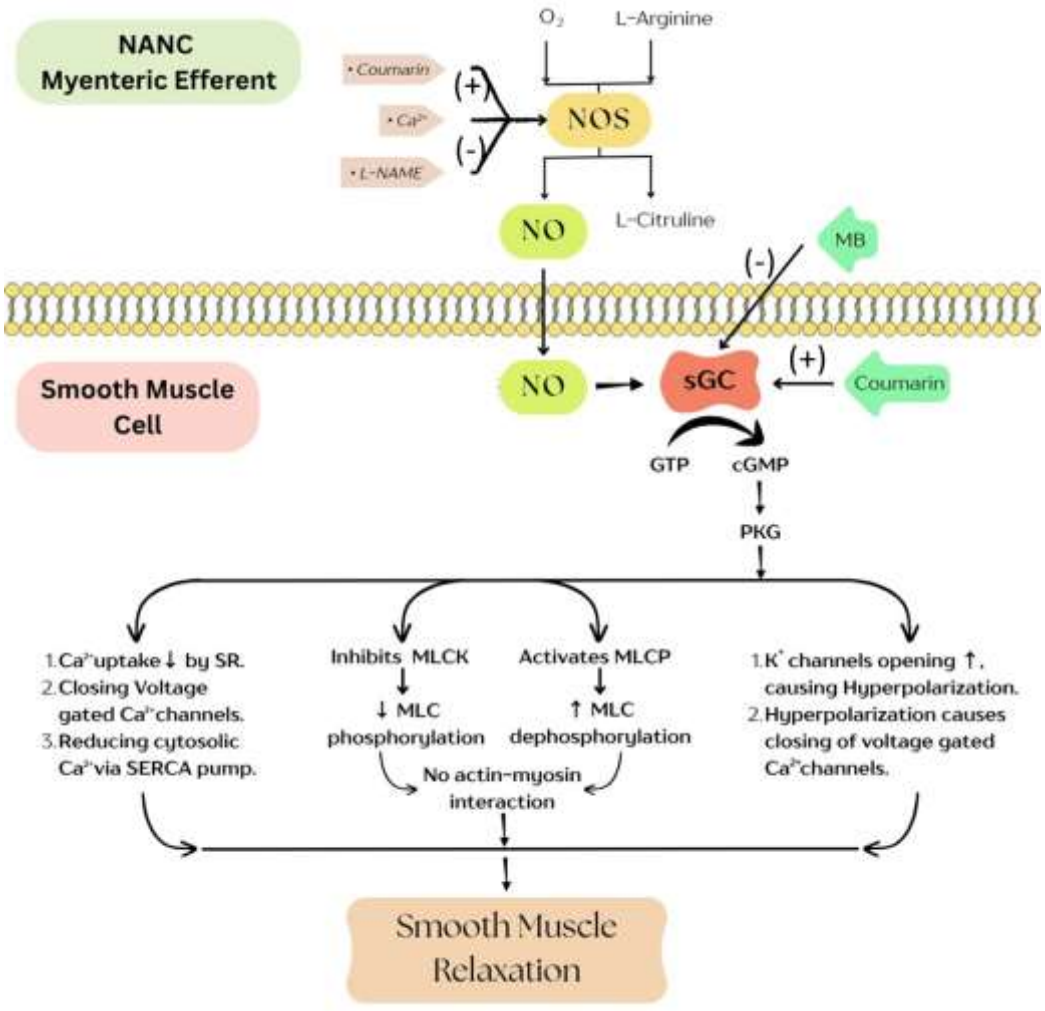

Figure 6. Showing the probable pharmacodynamic mechanism involved in the Coumarin induced inhibition of the contraction of the dVSM through inhibition of the activity of nitrergic intrinsic myenteric neurons. NO- nitric oxide; NOS- nitric oxide synthase; sGC- soluble guanylyl cyclase; cGMP-cyclic guanosine monophosphate; PKG- protein kinase G; [Ca^{2+}]- intracellular concentration; MLCK-myosin light chain kinase; MLCP-myosin light chain phosphatase; -, indicates inhibition/suppression; +, indicates increase/potentiation; ⇩, indicates decrease in activity or production; ⇧, indicates increase in activity or production.

CONCLUSION

In conclusion, the study suggests that Coumarin inhibits the contractile activity of duodenal visceral smooth muscle (dVSM) by suppressing the contractions of the smooth muscle within the duodenal wall, which motility to it and helps the small intestine to perform its digestive and absorptive functions. The findings indicate that the Coumarin induced inhibition of the dVSM contraction is likely mediated through the augmentation of the soluble guanylyl cyclase (sGC)-dependent nitrergic signaling pathway. These results further imply that chronic consumption of Coumarin may potentially impair duodenal motility, leading to disruptions in digestion and nutrient absorption in humans.

Declaration by Authors

Ethical Approval: Approved

Conflict of Interest: No conflicts of interest declared.

REFERENCES

1. Abraham, K., Wöhrlin, F., Lindtner, O., Heinemeyer, G., & Lampen, A. (2010). Toxicology and risk assessment of coumarin: focus on human data. *Molecular nutrition & food research*, *54*(2), 228-239. https://doi.org/10.1002/mnfr.200900281
2. Ballin, N. Z., & Sørensen, A. T. (2014). Coumarin content in cinnamon containing

food products on the Danish market. *Food Control*, *38*, 198-203. https://doi.org/10.1016/j.foodcont.2013.10.014

3. Borges, F., Roleira, F., Milhazes, N., Santana, L., & Uriarte, E. (2005). Simple coumarins and analogues in medicinal chemistry: occurrence, synthesis and biological activity. *Current medicinal chemistry*, *12*(8), 887-916. https://doi.org/10.2174/0929867053507315
4. Bult, H., Boeckxstaens, G. E., Pelckmans, P. A., Jordaens, F. H., Maercke, Y. V., & Herman, A. G. (1990). Nitric oxide as an inhibitory non-adrenergic non-cholinergic neurotransmitter. *Nature*, *345*(6273), 346-347. https://doi.org/10.1038/345346a0
5. Ghosh, R., Mukherjee, S., Sarkar, K., Paul, G. (2021). Potentiation of the contraction of duodenal visceral smooth muscle in rat through oxidative stress induced inhibition of AChE activity by methylparaben. Science Archives, Vol. 2 (3), 194-200. http://dx.doi.org/10.47587/SA.2021.2307
6. Hirano, I., Kakkar, R. A. H. U. L., Saha, J. K., Szymanski, P. T., & Goyal, R. K. (1997). Tyrosine phosphorylation in contraction of opossum esophageal longitudinal muscle in response to SNP. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *273*(1), G247-G252. https://doi.org/10.1152/ajpgi.1997.273.1.G247
7. Lake, B. G. (1999). Coumarin metabolism, toxicity and carcinogenicity: relevance for human risk assessment. *Food and chemical toxicology*, *37*(4), 423-453. https://doi.org/10.1016/S0278-6915(99)00010-1
8. Lungarini, S., Aureli, F., & Coni, E. (2008). Coumarin and cinnamaldehyde in cinnamon marketed in Italy: a natural chemical hazard? *Food additives and contaminants*, *25*(11), 1297-1305. https://doi.org/10.1080/02652030802105274
9. Murthy, K. S. (2006). Signaling for contraction and relaxation in smooth muscle of the gut. *Annu. Rev. Physiol.*, *68*(1), 345-374. https://doi.org/10.1146/annurev.physiol.68.040504.094707
10. Venugopala, K. N., Rashmi, V., & Odhav, B. (2013). Review on natural coumarin lead compounds for their pharmacological activity. *BioMed research international*, *2013*(1), 963248. https://doi.org/10.1155/2013/963248
11. Wang, Y. H., Avula, B., Nanayakkara, N. D., Zhao, J., & Khan, I. A. (2013). Cassia cinnamon as a source of coumarin in cinnamon-flavored food and food supplements in the United States. *Journal of agricultural and food chemistry*, *61*(18), 4470-4476. https://doi.org/10.1021/jf4005862
12. Wood, J. D. (1999). Sympathetic and Enteric Divisions of the Autonomic Nervous System. *Chinese Journal of Physiology*, *42*(4), 201-210.

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