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Coumarin-Induced Delay in Gastrointestinal Transit Through Facilitation of Nitrergic Neurotransmission in Male Albino Rats

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ABSTRACT

Coumarin, a natural plant-derived benzopyrone compound, is widely known for its anticoagulant, anti-inflammatory, and vasodilatory properties. Humans are often exposed to Coumarin through consumption of Coumarin tainted foods. Upon exposure, the small intestine gets primarily exposed to Coumarin, and it might exert its effects on the small intestine. Thus, the goal of this study was to examine the influence of Coumarin-induced changes in contractile activity of the small intestine *in vivo* by assessing gastrointestinal transit using the charcoal meal test. Our study indicated that animals exposed to Coumarin had significantly lowered gastrointestinal transit rates compared to control rats. Coumarin inhibits small intestinal transit by inhibiting the contractions of the visceral smooth muscles located in the muscularis externa layer of the small intestine that provides motility to the small intestine. However, intraperitoneal administration of L-NAME and Methylene Blue significantly reverse inhibitory effect of Coumarin on the gastrointestinal transit. In conclusion, it could be suggested that Coumarin may reduce gastrointestinal transit by inhibiting smooth muscle contractions at the small intestinal wall probably by activating nitrergic intrinsic myenteric efferents that secrete nitric oxide. These findings highlight the potential therapeutic application of coumarin in conditions associated with hypermotility and point to nitrergic signaling as its primary mechanism of action.

Keywords: Coumarin, Gastrointestinal transit, nitric oxide, L-NAME, nitrergic signaling.

INTRODUCTION

Naturally occurring plant-based compounds have attracted considerable attention due to their ability to influence physiological processes while generally causing fewer side effects than synthetic medications. Coumarin, a naturally occurring benzopyrone found in many plants such as *Dipteryx odorata*, cinnamon, and tonka beans, has been extensively studied for its wide range of pharmacological properties, including anticoagulant, anti-inflammatory, antioxidant, and vasodilatory effects (Lake, 1999; Venugopala et al., 2013). Coumarin is the foundational molecule for vital drugs like

warfarin (a vitamin K antagonist) (Abraham et al., 2010). Despite its clinical importance, there are growing worries about the health risks associated with taking in too much coumarin, especially from supplements or food contamination. Although many nations have banned coumarin as a food additive due to its potential to harm the liver (hepatotoxicity), people are still naturally exposed through spices and herbal products (Abraham et al., 2010; Lake, 1999). Many products today contain coumarin because they are spiced with Cassia cinnamon. Coumarin levels vary, with cinnamon sticks typically having more than ground cinnamon (Ballin and Sørensen, 2014). This presence in cinnamon is a significant concern, as research—including a study in Italy that found over 70% of tested cinnamon goods exceeded legal limits—shows that some products violate European regulatory standards (Lungarini et al., 2018; Wang et al., 2013).

While coumarin's therapeutic benefits are well-documented in the domains of cardiovascular and inflammatory disorders, its role in gastrointestinal physiology remains underexplored. The small intestine helps to digest food and absorb nutrients, a process regulated by smooth muscle contractions. These contractions move food along the digestive tract and mix it with enzymes. Coumarin is believed to enter the body through contaminated food and then circulates in the blood, damaging organs. It specifically harms the gastrointestinal tract, which impairs nutrient absorption. The researchers hypothesized that Coumarin intoxication would disrupt the smooth muscle contractions in the small intestine, thus altering how food moves through the small intestine. This change in motility would ultimately lead to poor digestion and malabsorption.

In order to examine the effect of Coumarin on the contractions of the SiVSM *in vivo,* the charcoal meal method was performed to measure the gastrointestinal transit time in living subjects. The primary goal of our study was to determine how Coumarin affects this transit time and, also the influence of Coumarin on the motor activity of the small intestine. The regulation of gastrointestinal (GI) motility is a complex physiological process involving coordinated smooth muscle contractions, enteric neuronal activity, and various neurotransmitters and signaling molecules. Disruptions in this process can result in significant clinical conditions such as diarrhea, irritable bowel syndrome (IBS), functional dyspepsia, and intestinal pseudo-obstruction (Knowles et al., 2017). Among the various modulators of intestinal motility, nitric oxide (NO) plays a pivotal role as a key inhibitory neurotransmitter in the enteric nervous system. NO is synthesized from L-arginine by nitric oxide synthase (NOS) and exerts its biological effects primarily through the activation of soluble guanylyl cyclase (sGC), leading to increased intracellular levels of cyclic guanosine monophosphate (cGMP). This cascade culminates in the relaxation of gastrointestinal smooth muscles and suppression of motility (Bult et al., 1990; Sanders et al., 2006).

The effects of coumarin on the Gastrointestinal Transit has not been reported till date. Therefore, the present study aims to evaluate the effect of coumarin on gastrointestinal transit in male albino rats and to elucidate the underlying mechanism with a focus on the nitrergic signaling pathway. By employing specific pharmacological modulators, this study investigates whether coumarin-induced changes in GI motility are mediated through the NO–cGMP axis. This research is expected to provide insights into the potential of coumarin as a natural therapeutic agent for managing gastrointestinal motility disorders, particularly those associated with hypermotility.

MATERIALS & METHODS

Chemicals and Reagents

The study utilized reagents and chemicals that were all of analytical grade. The chemicals used in the study include Coumarin procured from Sigma-Aldrich,

USA). Methylene blue, Charcoal meal (5% charcoal in 1% gum acacia) are purchased from E Merck, India, and N-ω-nitro-L-arginine methyl ester (L-NAME) hydrochloride is obtained from Sigma Aldrich, USA.

Experimental Animals and care

Adult male Sprague Dawley albino rats, weighing between 130 and 150 g and approximately two to three months old, were chosen as the experimental model. They were housed in the animal house in compliance with the rules set forth by the Kalyani University animal ethics committee, fed laboratory chow and water, and kept in a temperature range of 25 to 27°C in the departmental animal care room with a 24-hour light-dark cycle.

Experimental Design

The animals were treated to different exposure conditions as mentioned in Table 1. Rats were fasted for 18 hours prior to the experiment but had free access to water. Animals were randomly divided into 7 groups (n = 6/group):

Table 1. Experimental set up for the study.

Groups	Exposure condition
Set A	Control – Received distilled water
Set B	Received 20 µM Coumarin (Treated I)
Set C	Received 40 µM Coumarin (Treated II)
Set D	Received 80 µM Coumarin (Treated III)
Set E	Received 160 µM Coumarin (Treated IV)
Set F	Received 160 µM Coumarin (Treated IV) in L-NAME (10 mg/kg BW) intraperitoneally pre-treated condition
Set G	Received 160 µM Coumarin (Treated IV) in MB (1 mg/kg BW) intraperitoneally pre-treated condition

Sacrifice of the Animals

Prior to their sacrifice, the experiment's chosen animals were kept under fasting conditions for the whole night. The cervical dislocation procedure was used during the sacrifice in accordance with the protocols of Kalyani University's Animal Ethics Committee, in order to minimize suffering for the animals.

Charcoal meal test

The animals were allowed to starve for the duration of the night. After administering the test chemical, using an oral feeding needle, each rat in a group is given a suspension of 0.5 mL charcoal meal (10% w/v wood charcoal in 5% w/v gum acacia aqueous suspension). Twenty minutes later, the animals were sacrificed by cervical dislocation, the abdomen is cut open, and the marker's leading edge is located. To stop the peristalsis, the leading edge of the intestine is knotted with cotton thread, or the entire intestine-from the stomach's pyloric end to the ileocecal junction-is instantly submerged in 5% formalin solution. Both the length of the intestine overall and the distance covered by the leading edge of charcoal are measured. The entire segment of the small intestine, beginning at the pyloric end, was put on the blotting paper. To prevent any harm to the intestines, every precaution was taken. The specific distance, traversed by the charcoal meal was estimated which is represented as the percentage of the Gastro-intestinal transit. The percentage of the Gastro-intestinal transit was measured by following the under mentioned formula:

$$\text{GI Transit } (\%) = \left(\frac{\text{Distance traveled by charcoal}}{\text{Total length of small intestine}} \right) \times 100$$

STATISTICAL ANALYSIS

All data were presented as mean ± standard error of the mean (SEM). Statistical analysis was conducted using one-way analysis of variance (ANOVA) with GraphPad Prism software (version 8). Differences between

groups were considered statistically significant when the p-value was less than 0.05 ($P < 0.05$).

RESULTS AND DISCUSSION

To investigate the gastrointestinal toxicity induced by coumarin, we evaluated its effect on gastrointestinal (GI) transit as an indicator of intestinal motility *in vivo*. Using the charcoal meal test, we observed that oral administration of coumarin significantly reduced GI transit in a dose-dependent manner, expressed as a percentage change in charcoal propulsion (Figure 1 and Table 2). Since small intestinal motility plays a crucial role in propelling luminal contents toward the anus through coordinated peristaltic contractions, our findings suggest that the coumarin-induced delay in GI transit is likely a result of inhibited contraction of the visceral smooth muscle located in the muscularis externa. This inhibition may be attributed to the impaired contractility of small intestinal visceral smooth muscle (SiVSM) due to coumarin-induced toxicity.

We hypothesize that the coumarin-induced suppression of motility, which contributes to delayed transit, might be due to inactivation of myenteric excitatory efferents (primarily cholinergic neurons releasing acetylcholine) and/or enhanced activity of inhibitory pathways. These could include adrenergic myenteric neurons releasing epinephrine or, more notably, nitrergic (non-adrenergic, non-cholinergic; NANC) neurons that release nitric oxide (NO)—a principal inhibitory neurotransmitter that mediates smooth muscle relaxation.

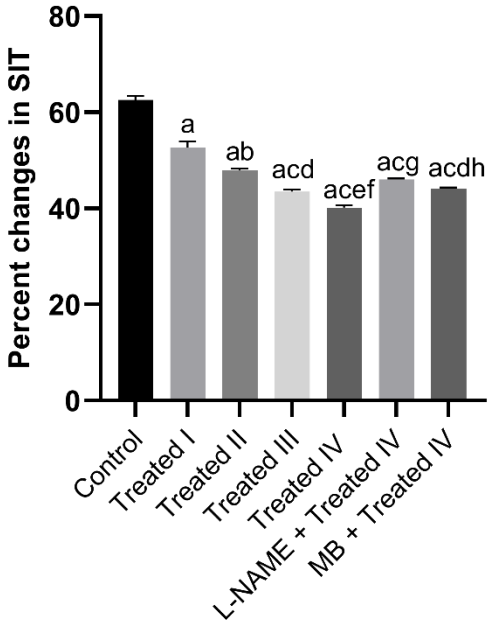

Figure 1. Bar diagram showing percent changes in gastrointestinal transit as a result of the Coumarin induced effects on the contractions of the small intestine. The data represented were mean ± SEM for all the group. [a]*p*<0.0001 vs. Control, [b,c]*p*< 0.01, 0.0001 vs. Treated I, [d,e]*p*< 0.01, 0.0001, vs. Treated II, [f]*p*<0.05 vs. Treated III and [g,h]*p*<0.001, 0.01 vs. Treated IV.

However, the specific involvement of nitrergic pathways in coumarin's action on small intestinal transit has not been systematically studied. Given that the NO–cGMP pathway is central to inhibitory neuromuscular signaling in the GI tract, it is plausible that coumarin exerts its effects on intestinal transit through modulation of this pathway. L-NAME (N^G^-nitro-L-arginine methyl ester), a non-selective NOS inhibitor and methylene blue (an sGC inhibitor), provide a valuable framework to investigate the role of the nitrergic system in small intestinal transit. Further to explore the

possible neurocrine mechanism underlying coumarin's effects, we assessed GI transit in rats pre-treated with L-NAME (a nitric oxide synthase inhibitor, 10 mg/kg body weight) and methylene blue (a soluble guanylyl cyclase inhibitor, 1 mg/kg body weight), both administered intraperitoneally before coumarin exposure. The results demonstrated that the extent of coumarin-induced delay in GI transit was significantly attenuated under both L-NAME and methylene blue pre-treated conditions, compared to coumarin treatment alone.

These findings suggest that the inhibitory effect of coumarin on small intestinal motility is, at least in part, mediated through activation or enhancement of the nitrergic myenteric efferent pathway. Specifically, the data support the involvement of nitric oxide and its downstream effector, soluble guanylyl cyclase, in mediating smooth muscle relaxation and the consequent suppression of small intestinal transit.

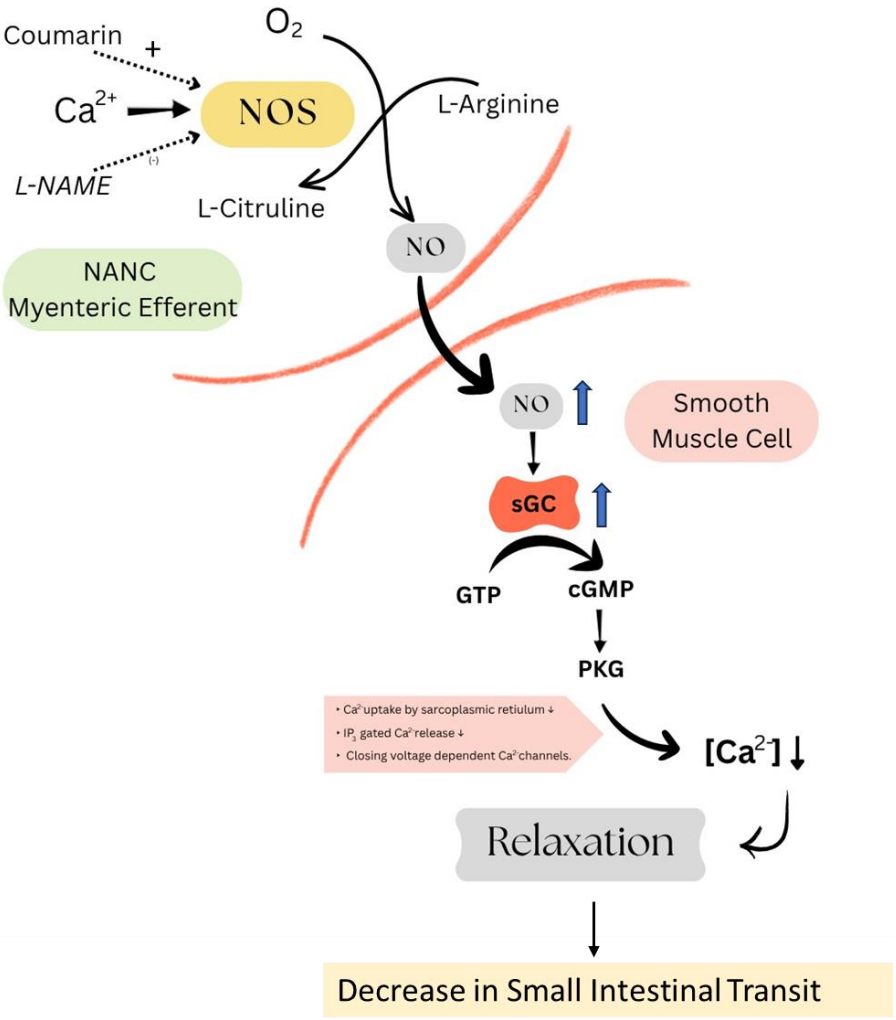

Figure 2. Schematic representation of the probable neurocrine mechanisms involved in the Coumarin induced decrease in the gastrointestinal transit. NO- nitric oxide; NOS- nitric oxide synthase; sGC- soluble guanylyl cyclase; cGMP-cyclic guanosine monophosphate; PKG- protein kinase G; $[Ca^{2+}]$- intracellular concentration. (+), indicates stimulation; (-), indicates inhibition; ↓ indicates decrease in levels, ↑ indicates increase in levels.

CONCLUSION

Coumarin decreases the gastrointestinal transit by suppressing the contractile activity of the SiVSM through inhibition of the contractions of the smooth muscle located at the muscularis externa layer of the small intestine. Further, the Coumarin induced suppression of the contractile activity of the

SiVSM is due to activation of intrinsic nitrergic myenteric efferents that promotes relaxation of the SiVSM and results in delayed gastrointestinal transit. Thus, Coumarin may have therapeutic potential in treating conditions of hypermotility and diarrhea like gastrointestinal disorders.

Declaration by Authors

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