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Tartrazine Promotes the Contraction of the Duodenal Visceral Smooth Muscle by Facilitating the Cholinergic Signalling Pathway

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ABSTRACT

Tartrazine (TAZ), a synthetic azo dye, continues to be used widely in the food industry for its bright yellow coloration, despite being restricted in several countries. Chronic exposure to TAZ through contaminated food raises concerns regarding its impact on gastrointestinal physiology. This study investigates the effect of Tartrazine on the contractile function of the small intestinal visceral smooth muscle. *Ex vivo* recordings of duodenal contractions were obtained using an isotonic transducer (IT-2245) coupled with the RMS Polyrite D data acquisition system. Tartrazine-treated rat duodenal tissues displayed a significant increase in both the amplitude and frequency of spontaneous contractions in a dose dependent manner. To understand the neurogenic basis of this augmentation, the tissues were co-treated with cholinergic agonists (e.g., Acetylcholine) and antagonists such as Atropine (a cholinergic receptor blocker). The enhanced contractility induced by Tartrazine was significantly attenuated by Atropine, indicating a muscarinic receptor-mediated mechanism. The results suggest that Tartrazine augments the contractile activity of duodenal visceral smooth muscle (dVSM) probably by potentiating the cholinergic signalling pathways. This excitatory effect may be due to increased acetylcholine release or enhanced muscarinic receptor sensitivity, ultimately contributing to increased motility upon exposure.

Keywords: Tartrazine, Duodenal visceral smooth muscle (dVSM), Gastrointestinal motility.

INTRODUCTION

The global food industry has undergone a significant transformation in recent decades, with the incorporation of synthetic additives, particularly artificial colorants, to improve the visual appeal and marketability of food products. Among these, Tartrazine (E102 or FD&C Yellow No. 5) is one of the most commonly used azo dyes, providing a bright yellow hue that appeals to consumers, especially in soft drinks, desserts, snacks, pharmaceuticals, and cosmetics (Kobylewski & Jacobson, 2012; EFSA, 2009). Its widespread use is attributed to its water solubility, chemical stability, and low

production cost. Despite these advantages, concerns about the potential toxicity of Tartrazine have led several countries to regulate or ban its use (Amin, & Al-Shehri, 2018; Waly et al., 2022). Multiple studies have linked chronic exposure to Tartrazine with hypersensitivity reactions, urticaria, asthma, and behavioral disorders such as ADHD in children (McCann et al., 2007; Waly et al., 2022; Batada & Jacobson, 2016). Consequently, the toxicological evaluation of Tartrazine has become a key issue in public health, particularly regarding its long-term effects on physiological systems. While its systemic toxicity—including hepatotoxicity, nephrotoxicity, and reproductive toxicity—has been well-documented (Wahab and Moram, 2013; Visternicu et al., 2025), very limited studies have examined the influence of Tartrazine on small intestinal motility, a critical determinant of digestive efficiency and nutrient absorption. The duodenum, the first part of the small intestine, plays a pivotal role in coordinating peristaltic movement, hormone release, and chyme neutralization. Its activity is governed by myogenic mechanisms and intricate neurogenic control, predominantly mediated by the enteric nervous system (ENS) (Furness, 2012).

Although Tartrazine has traditionally been considered inert in the digestive tract, emerging evidence suggests that it may exert pharmacodynamic actions on smooth muscle either directly or via modulation of neurochemical pathways. This gap in knowledge holds significant implications for understanding diet-induced changes in the SiVSM contractions and their contribution to chronic gastrointestinal complaints such as cramps, bloating, and altered bowel habits. Therefore, the primary aim of this study is to evaluate the effect of Tartrazine on duodenal visceral smooth muscle (dVSM) contractile function in rats *ex vivo* using an isotonic transducer. This model provides a controlled physiological setting to analyse contraction amplitude and frequency. Furthermore, the study investigates whether muscarinic cholinergic receptors mediate Tartrazine's effect by employing acetylcholine (a muscarinic agonist), Atropine (a muscarinic antagonist). By elucidating the possible cholinergic modulation of Tartrazine-induced duodenal contractions, this study contributes to the emerging field of neuroenteric toxicology and highlights the need for re-evaluating the safety of widely used but poorly understood food additives.

MATERIALS & METHODS

Chemicals and Reagents

All chemicals and reagents used in this study were of analytical grade. Tartrazine (≥98% purity), the primary test compound, was procured from Sigma-Aldrich. Additional chemicals, including acetylcholine chloride (ACh), atropine sulfate (a muscarinic receptor antagonist), sodium chloride (NaCl), potassium chloride (KCl), magnesium chloride ($MgCl_2$), calcium chloride ($CaCl_2$), sodium bicarbonate ($NaHCO_3$), sodium dihydrogen phosphate (NaH_2PO_4), and glucose, were sourced from E. Merck, India. All solutions were freshly prepared prior to use and aerated with a gas mixture of 95% O_2 and 5% CO_2.

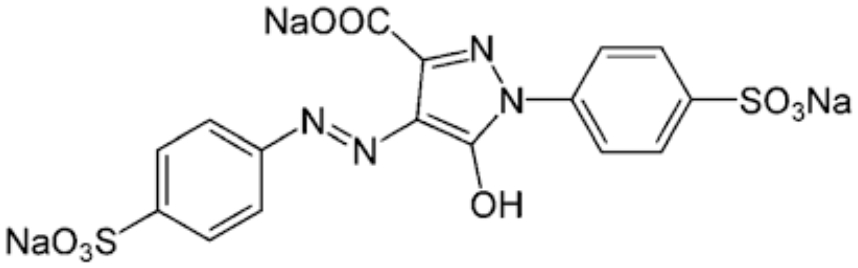

Figure1: Chemical structure of tartrazine

Experimental Animals and care

Adult Sprague Dawley albino rats, weighing between 130 and 150 grams and aged 2 to 3 months, were selected for this study. The animals were maintained in the departmental animal house under standard laboratory conditions, including a temperature-controlled environment (25–27°C) and a 12-hour light/dark cycle. They were fed standard laboratory chow and had free access to water throughout the study period. All procedures involving animals were carried out in strict adherence to the ethical guidelines approved by the Animal Ethics Committee, University of Kalyani.

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	Received 0.3 mM TAZ (Treated I)
Set 2	Received 0.6 mM TAZ (Treated II)
Set 3	Received 0.9 mM TAZ (Treated III)
Set 4	Received 1.2 mM TAZ (Treated IV)
Set 5	Received 0.01 µM Acetylcholine
Set 6	Received 0.9 mM TAZ in presence of 0.01 µM Acetylcholine
Set 7	Received 1 µM Atropine
Set 8	Received 0.9 mM TAZ in presence of 1 µM Atropine

Animal Sacrifice

The animals selected for the experiment were subjected to overnight fasting prior to sacrifice to standardize physiological conditions. Cervical dislocation was employed as the method of euthanasia to ensure minimal pain and distress, in accordance with the ethical guidelines approved by the Animal Ethics Committee of the University of Kalyani.

Recording of Duodenal Movement

To evaluate the spontaneous contractile activity of duodenal visceral smooth muscle (dVSM) ex vivo, a duodenal segment approximately 3 cm in length was carefully excised and vertically suspended in an organ bath containing 40 mL of freshly prepared Tyrode's solution. The tissue was mounted using two stainless steel hooks inserted at either end of the segment—one attached to the base of the organ bath and the other connected to the lever arm of an isotonic transducer (Model IT-2245). The Tyrode's solution used had the following composition per liter: NaCl – 8.0 g, KCl – 0.2 g, $CaCl_2$ – 0.2 g, $MgCl_2$ – 0.1 g, NaH_2PO_4 – 0.05 g, $NaHCO_3$ – 1.0 g, and dextrose – 1.0 g, with the pH adjusted to 7.4. Tissue viability was maintained by continuously aerating the bath with a gas flow delivering 2–3 oxygen bubbles per second via an oxygen bubbler. The bath temperature was precisely regulated at 37 ± 0.5°C using an automatic thermostat integrated into a Dale's apparatus. Contractile responses of the tissue were recorded using the isotonic transducer, which was interfaced with a data acquisition system and analysis software (RMS Polyrite-D, RMS, Chandigarh, India) to continuously monitor mechanical activity. Each tissue segment was allowed an equilibration period of at least 35 minutes under experimental conditions, during which it was regularly washed with fresh Tyrode's solution to remove any accumulated metabolites. After stabilization, spontaneous isotonic contractions were recorded continuously, followed by analysis of the tissue responses to graded concentrations of Tartrazine (TAZ) and selected pharmacological blockers.

STATISTICAL ANALYSIS

Data from each experimental group were expressed as the mean ± standard error of the mean (SEM). The frequency and

amplitude of duodenal contractions were analyzed to determine the contractile force. For functional assessments, data from treated tissues were expressed as percentage changes relative to their corresponding basal (control) values. Statistical comparisons between groups were performed using one-way analysis of variance (ANOVA) in GraphPad Prism (version 8). A p-value of less than 0.05 ($P < 0.05$) was considered indicative of statistical significance.

RESULTS AND DISCUSSION

Effect of graded doses of Tartrazine (TAZ) on spontaneous duodenal contractions Ex Vivo in rats

To evaluate the impact of Tartrazine (TAZ) on the contractile activity of duodenal visceral smooth muscle (dVSM), *ex vivo* recordings of duodenal motility were performed using graded concentrations of TAZ in single-dose acute exposure experiments. Administration of Tartrazine resulted in a dose-dependent increase in the amplitude and frequency of spontaneous duodenal contractions. From the tracings it could be hypothesised that the increase in the contraction of the dVSM by TAZ might be due to augmentation in the activity of excitatory cholinergic myenteric efferents and/or inhibition of the inhibitory nitrergic and/or adrenergic efferents innervating the dVSM (Figure 2 and Figure 3).

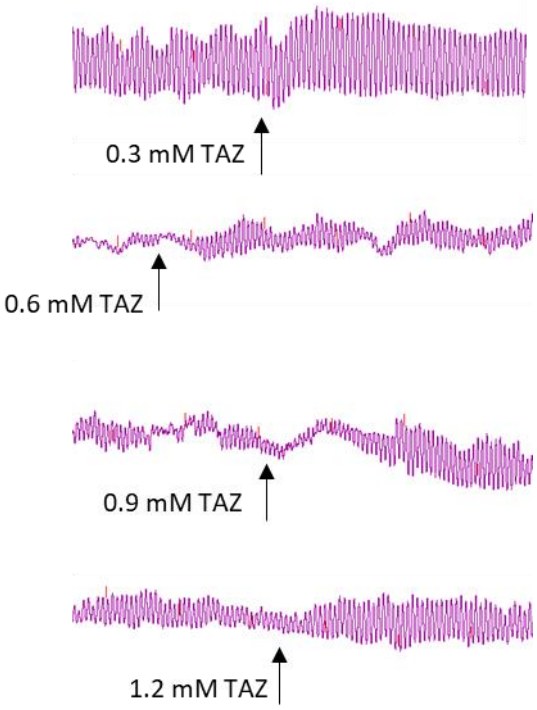

Figure 2. Tracings showing representative records of the effect of graded concentrations of TAZ on the isolated duodenal segment in order to examine the effect of TAZ on the contraction of the SiVSM in rat *ex vivo* obtained with an isotonic transducer coupled to RMS Polyrite-D.

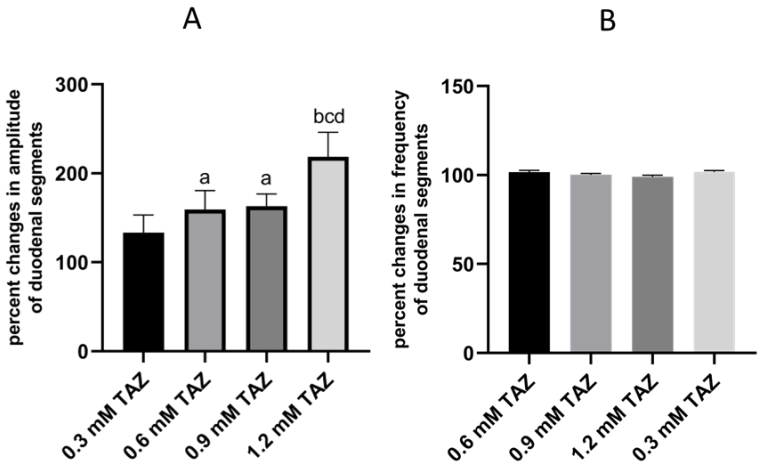

Figure 3. Bar diagrams showing the percent changes in the amplitude (A) and frequency (B) of contraction of the duodenum in TAZ exposed groups compared to control. The data were represented as mean ± SEM for all the groups. [a,b]*p*<0.01, 0.0001 vs. 0.3 mM TAZ, [c]*p*< 0.0001 vs. 0.6 mM TAZ and [d]*p*<0.0001, vs. 0.9 mM TAZ (A).

Effect of Tartrazine (TAZ) on the Duodenal Movements Pre-Incubated with Acetylcholine

Exogenous administration of Acetylcholine (0.01 µM) independently increased the frequency and amplitude of duodenal contractions, similar to the effect observed with Tartrazine. Further, application of TAZ in presence of ACh produce synergistyic effects (P > 0.05 compared to ACh alone). This supports the hypothesis that Tartrazine may exert its facilitatory effects on the contraction of the dVSM by modulating the cholinergic neurotransmission mechanism.

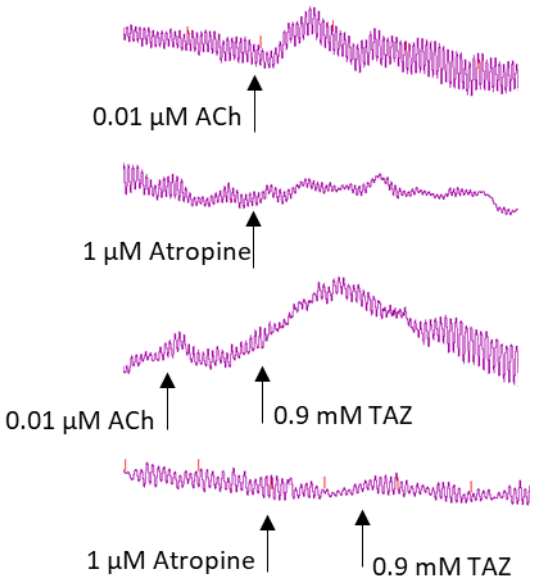

Figure 4. Tracings showing representative records of the effect of TAZ on the isolated duodenal segment in response to the application of cholinergic agonist and antagonist in order to examine the neurocrine mechanism involved in the TAZ induced on the contraction of the SiVSM in rat *ex vivo* obtained with an isotonic transducer coupled to RMS Polyrite-D.

Effect of Tartrazine (TAZ) on the Duodenal Movements Pre-Incubated with Atropine

Pre-incubation with Atropine sulfate (1 µM), a cholinergic receptor antagonist, markedly attenuated the Tartrazine-induced

enhancement of the contraction of the dVSM. In the presence of atropine, the contractile response to Tartrazine was reduced compared to the effect of Tartrazine alone was statistically significant ($P < 0.05$). This suggests a significant involvement of muscarinic cholinergic receptors in the excitatory effect of Tartrazine on duodenal visceral smooth muscle (Figure 4 and Figure 5).

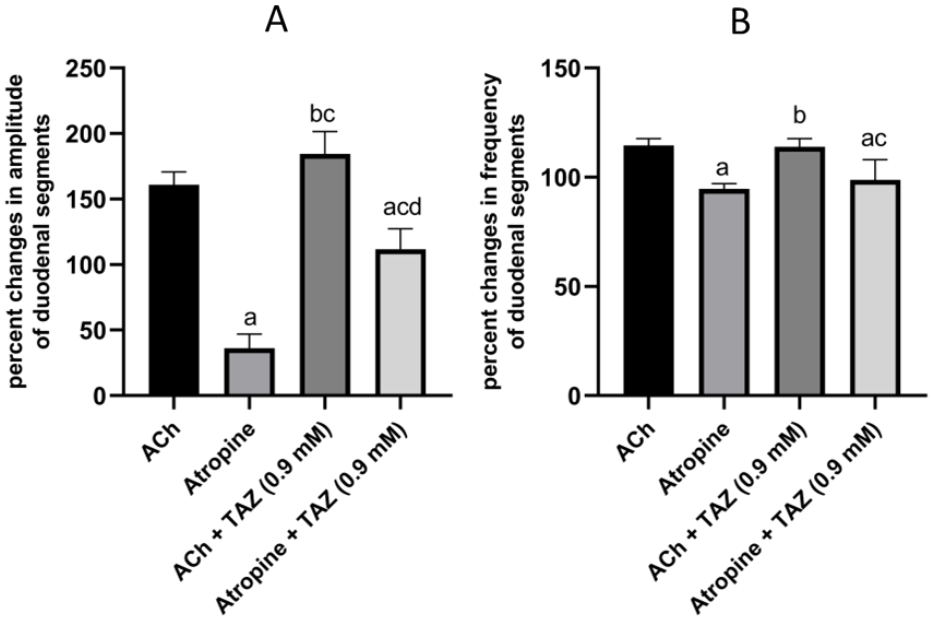

Figure 5. Bar diagrams showing the percent changes in the amplitude (A) and frequency (B) of contraction of the duodenum in response to the application of TAZ in presence of ACh and Atropine (Cholinergic agonist and antagonist). The data were represented as mean ± SEM for all the groups). [a,b]*p*<0.0001, 0.001 vs. ACh, [c]*p*< 0.0001 vs. Atropine, [d]*p*<0.0001 vs. ACh + TAZ (0.9mM) (A). [a]*p*<0.0001 vs. ACh, [b]*p*< 0.0001 vs. Atropine, [c]*p*<0.0001 vs. ACh + TAZ (0.9mM) (B).

These results suggested that TAZ promotes the contraction of the dVSM probably through facilitation of the cholinergic myenteric activity by releasing ACh at the neuromuscular junction (Figure 6).

The duodenum, the first part of the small intestine, is essential for regulating intestinal contractions (peristalsis), releasing hormones, and neutralizing stomach acid. The small intestine's movement, or motility, is controlled by its own muscle rhythms and the enteric nervous system (ENS) (Furness, 2012). These movements, powered by contractions of the visceral smooth muscle in the intestinal wall, are crucial for proper digestion and nutrient absorption. When food additives or other substances interfere with these muscle contractions, it alters the small intestine's motility and transit time, which can impair both digestion and absorption. Recent research suggests that environmental toxins, such as food dyes and agricultural chemicals, can disrupt the nervous system of the gut. Specifically, these substances can interfere with cholinergic neurotransmission—a key process for muscle control—by affecting the release of the neurotransmitter acetylcholine (ACh), altering how sensitive its receptors are, or changing the activity of the enzyme that breaks it down (acetylcholinesterase) (Wopara et al., 2021). The food dye Tartrazine is a notable example. When broken down by gut microbes, it forms aromatic amines that can be neurotoxic, potentially affecting smooth muscle function and ENS signalling. Tartrazine has also been shown to cause oxidative stress, which can further damage neural excitability and neurotransmitter function in the gut (Biswas et al., 2023). While Tartrazine was once considered harmless to

the digestive tract, this study shows it can affect the function of the GI smooth muscle, possibly by interacting with the intrinsic nerves of the gut.

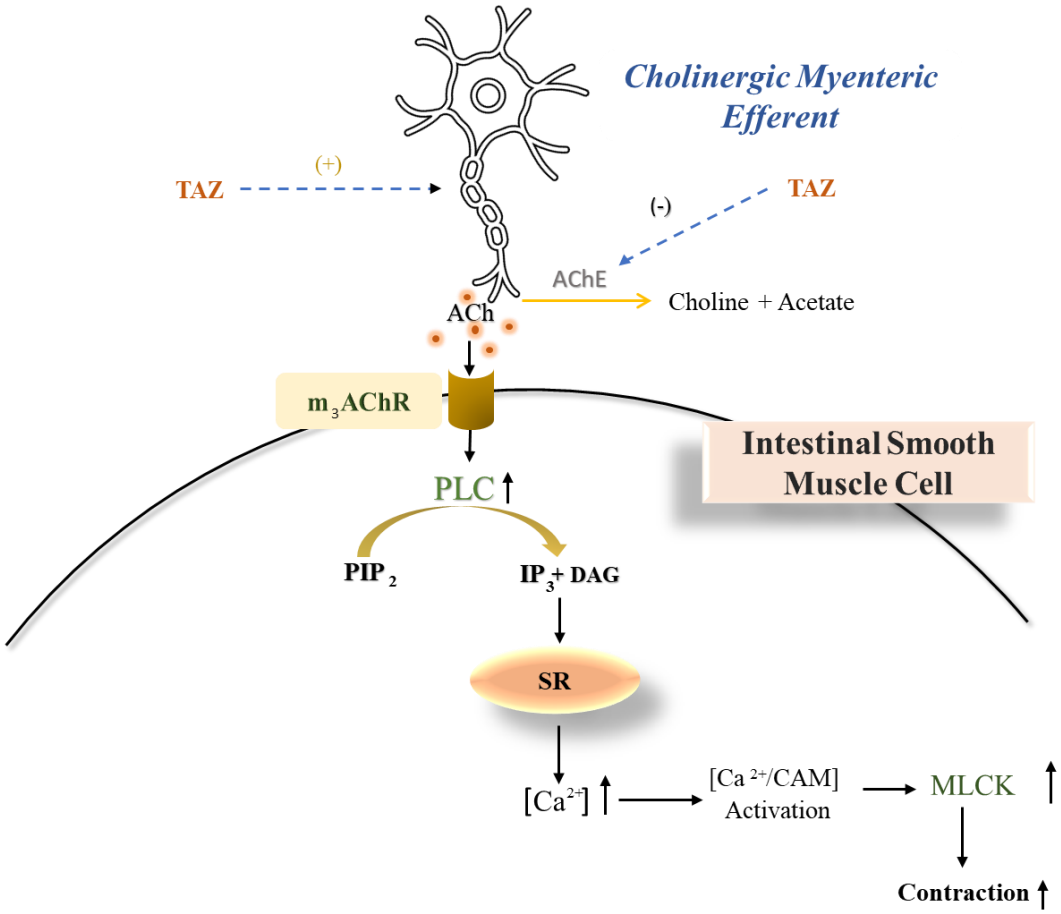

Figure 6. Schematic representation showing the probable mechanisms involved in the potentiation of the dVSM contraction by TAZ. TAZ- Tartrazine; ACh-acetylcholine; AChE- Acetylcholinesterase; [Ca^{2+}]- Intracellular calcium concentration; PIP_2 - Phosphatidylinositol 4,5-bisphosphate; PLC- Phospholipase C; IP_3- inositol 1,4,5-trisphosphate; DAG- Diacylglycerol; MLCK- myosin light chain kinase. +, indicates facilitation; -, indicates inhibition. ↑, indicates increase in production/activity.

Acetylcholine (ACh) acts as the main excitatory neurotransmitter in the gut, primarily stimulating muscle contraction by binding to muscarinic M3 receptors on the visceral smooth muscle. This process triggers an increase in intracellular calcium levels, which ultimately leads to muscle contraction (Johnson, 2006). The result of our study suggests that Tartrazine might facilitate the activity of excitatory cholinergic myenteric efferents that releases ACh at the synapse enpassant; and potentiates the contraction of SiVSM probably by elevating the intracellular calcium concentration through increased production of IP_3 (Inositol 1,4,5-triphosphate).

CONCLUSION

The findings of this study demonstrated that Tartrazine significantly enhances the spontaneous contractility of duodenal visceral smooth muscle in rats through modulation of the cholinergic signalling pathway. The results obtained from this study could be extrapolated in human beings. TAZ should be considered as an entero-toxicant. Daily intake of TAZ might alter the contractile function of the dVSM

leading to impaired digestion and improper absorption.

Declaration by Authors

Ethical Approval: Approved
Acknowledgement: None
Source of Funding: None
Conflict of Interest: The authors declare no conflict of interest.

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