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Research Article

Metanil Yellow Decreases the Gastrointestinal Transit through Suppression of the Contractile Activity of the Small Intestine

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ABSTRACT

Metanil yellow (MY), an unauthorized synthetic food color, is used in various food products because it is cheaper and more stable in color compared to natural coloring agents. Despite its prohibition, humans are still exposed to toxic MY through consuming contaminated food. While MY intoxication has been observed in various animal organ systems, its potential role in altering the contractile activity has not been examined till date. Thus, the purpose of this study was to investigate the impact of MY induced alteration in the contractile activity through assessment of the gastrointestinal transit by charcoal meal test. We have found significant decrease in the percent in gastrointestinal transit in MY exposed groups of rats compared to control rats. Moreover, significant counteractions of the MY induced inhibitory effect on the gastrointestinal transit have been observed in presence of L-NAME and Methylene Blue respectively. In conclusion, it can be suggested that MY induced decrease in the gastrointestinal transit might be due to inhibition of the contractions of the smooth muscle located at the wall structure of the small intestine that provides motility; through facilitation of the activity of nitrergic intrinsic myenteric efferents secreting nitric oxide, the prinicipal inhibitory neurotransmitter.

Keywords: Metanil yellow, contractile activity, charcoal meal test, nitrergic intrinsic myenteric efferents, L-NAME, Methylene Blue.

INTRODUCTION

In today's world, various synthetic colorants are added to food to make them more appealing to consumers. Some food colors are allowed within certain limits, while others are not permitted at all but are still used due to their stability and affordability compared to approved food colors. Metanil Yellow (MY) is a commonly used food colorant, known for its bright yellow color, and is used in sweets, spices, and other food products[1].

The synthesis of MY involves the diazotization of metanilic acid and diphenylamine[2], resulting in a yellow azo dye. Although it is permitted for use in industries to color wool, nylon, silk, paper, aluminum, ink, and detergent[3], MY is not approved as a food color under the Prevention of Food Adulteration Act 1954, India. Numerous studies have determined that MY is a significant toxic substance. It has been observed that MY can disrupt the normal secretion of neurotransmitters, cause harm to the developing brain, and hinder learning ability by damaging the granular and purkinjee cell layer[4]. Exposure to MY leads to the degeneration of tubular epithelium, disruption of Bowman's capsule, and swelling of renal tubular epithelial cells, causing harmful changes in the convoluted tubule and collecting duct[5]. MY has been shown to disturb normal estrous cycles and folliculogenesis in female rats by inducing oxidative stress[1]. In males, MY leads to the degeneration of seminiferous tubules[6]. Several studies have indicated that MY can trigger oxidative stress in essential organs such as the heart, kidney, and liver[7,8]. MY is known to have carcinogenic and mutagenic properties[9,10].

Despite being classified as a non-permitted food color, MY is widely employed for coloring various food items such as laddoo, biryani, and jalebi. Consequently, human consumption of MY-tainted foods leads to frequent exposure to this substance. The gastrointestinal system is the primary organ affected by MY upon ingestion of contaminated food, potentially causing toxicity and disrupting the normal physiological function of the digestive system.

The gastrointestinal system is responsible for the digestion of foods and the absorption of nutrients. This process is regulated by contractions of the gastrointestinal smooth muscle, which creates motility and facilitates mixing and churning of the chyme with enzymes. Additionally, it helps propel the contents of the intestines forward. It is believed that MY enters the body through the absorption of contaminated food by the intestine, and then it is carried to the blood. Subsequently, the toxic substance affects various organs and disrupts their metabolic processes. MY is known to hinder nutrient absorption by causing damage to the structural integrity of the gastrointestinal tract[11]. Thus, any disruption in the contractions of the visceral smooth muscle due to intoxication caused by MY will undoubtedly change the motor activity of the small intestine and will alter the gastrointestinal transit of the chyme that result in impaired digestion and malabsorption. The gastrointestinal motility is assessed *in vivo* with through determination of the gastrointestinal transit by charcoal meal method. Further, any alteration in transit time due to MY-induced intoxication is a clear indicator of impaired motility (motor activity) in the

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smooth muscle of the small intestinal viscera. Hence, the purpose of this research was to investigate how MY impacts gastrointestinal transit and its influence on the motor activity of the SiVSM.

MATERIALS AND METHODS

Chemicals and Reagents

The study utilized reagents and chemicals of analytical grade. The following chemicals were purchased from Sigma Aldrich, USA: Metanil Yellow, which is also known as 3-((4-(Phenylamino) phenyl) azo) benzenesulfonic acid monosodium salt, and N-ω-nitro-L- arginine methyl ester (L-NAME) hydrochloride. Methylene blue, gum acacia, and charcoal were bought from E-Merck in India.

Animals

The experimental model comprised adult female Sprague Dawley albino rats, with a weight ranging from 130 to 150 g and an age of approximately two to three months. They were accommodated in the animal house, adhering to the regulations established by the Kalyani University animal ethics committee. The rats were provided with laboratory chow and water, and they were kept in a departmental animal care room with a temperature maintained between 25 and 27°C and a 24-hour light-dark cycle.

Experimental Design

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

Table 1: Experimental Setup for the study

Groups	Exposure conditions
Group 1	Application of 8µM MY orally
Group 2	Application of 16µM MY orally
Group 3	Application of 32µM MY orally
Group 4	Application of single dose of L-NAME (10 mg/kg BW) intraperitoneally
Group 5	Application of single dose of MB (1 mg/kg BW) intraperitoneally
Group 6	Application of 32µM MY orally in L-NAME (10 mg/kg BW) intraperitoneally pre-treated condition
Group 7	Application of 32µM MY orally in MB (1 mg/kg BW) intraperitoneally pre-treated condition

Animal Sacrifice

The experiment's selected animals were fasted for the entire night before being sacrificed. In compliance with the guidelines established by Kalyani University's Animal Ethics Committee, the cervical dislocation technique was employed during the sacrifice to reduce the animals' pain to the least.

Charcoal Meal Test

The animals selected for the experiment were fasted for the entire night before being sacrificed. To reduce the animals' suffering during the sacrifice, the cervical dislocation technique was employed in compliance with Kalyani University's Animal Ethics Committee's guidelines. For the entire night, the animals were left to starve. Each rat in a group receives 0.5 mL of a suspension of charcoal meal (10% w/v wood charcoal in 5% w/v gum acacia aqueous suspension) via an oral feeding needle after the test chemical has been administered. The animals were sacrificed by cervical dislocation twenty minutes later, the abdomen was cut open, and the leading edge of the marker was found. The leading edge of the intestine can be knotted with cotton thread to stop the peristalsis, or the intestine can be immediately immersed in 5% formalin solution from the pyloric end of the stomach to the ileo-caecal junction. It is measured how long the intestine is overall as well as how far the leading edge of charcoal travels. Starting at the pyloric end, the whole small intestine segment was placed on the blotting paper. Every safety measure was done to ensure that the intestines would not be harmed. The estimated distance covered by the charcoal meal is expressed as a percentage of the transit through the gastrointestinal tract[12].

The following formula was used to calculate the percentage of gastrointestinal transit:

$$\% \text{ GIT} = \frac{distance\ travelled\ by\ the\ charcoal}{total\ length\ of\ the\ small\ intestine} \times 100$$

Statistical Analysis

The mean ± SEM was used to represent the values. The software GraphPad Prism 8 was used to perform a one-way ANOVA to examine the differences between the mean values in the groups of each drug treatment and the control. *P*< 0.05 was regarded as significant.

RESULTS AND DISCUSSION

We have investigated the impact of MY on gastrointestinal transit as a measure to evaluate gastrointestinal motility in vivo in order to investigate the MY-induced gastrointestinal toxicity. We found that MY, when taken orally, significantly reduced the gastrointestinal transit in a dose-response manner (measured as the percent change in the gastrointestinal transit) using the charcoal meal test. The results clearly show that the MY-induced decrease in gastrointestinal transit is caused by inhibition of small intestinal motility, which is caused by decreased contraction of the visceral smooth muscle found in the muscularis externa of the small intestine in response to MY-induced intoxication of the small intestinal visceral smooth muscle (SiVSM). This is because we know that the motility of the small intestine aids in propulsion of the food towards anus through its peristaltic movement. The inactivation of excitatory cholinergic myenteric efferents that secrete acetylcholine (ACh) and/or enhancement of the activity of inhibitory adrenergic myenteric efferents releasing epinephrine or enhancement of the activity of

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inhibitory nitrergic (NANC, non-adrenergic non cholinergic) myenteric efferents that release Nitric Oxide (NO), the primary neurotransmitter responsible for smooth muscle relaxation, may be the cause of the MG-induced suppressed motility that led to delayed gastrointestinal transit.

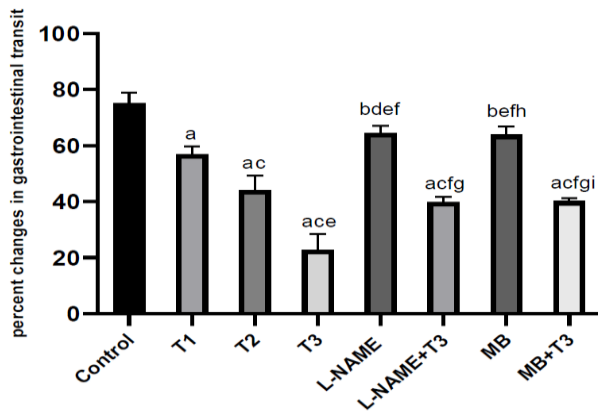

Figure 1: Bar diagram showing percent changes in gastrointestinal transit as a result of the MY induced effects on the contractions of thesmall intestine. The data represented were mean ± SEM for all the group. $^{b,a}P$<0.001, 0.0001 Vs Control; $^{d,c}P$<0.05, 0.0001Vs T1; ^{e}P<0.0001 Vs T2; ^{f}P<0.0001 Vs T3; ^{g}P<0.0001 Vs L-NAME; ^{h}P<0.0001 Vs L-NAME+T3; ^{i}P<0.0001 Vs MB.

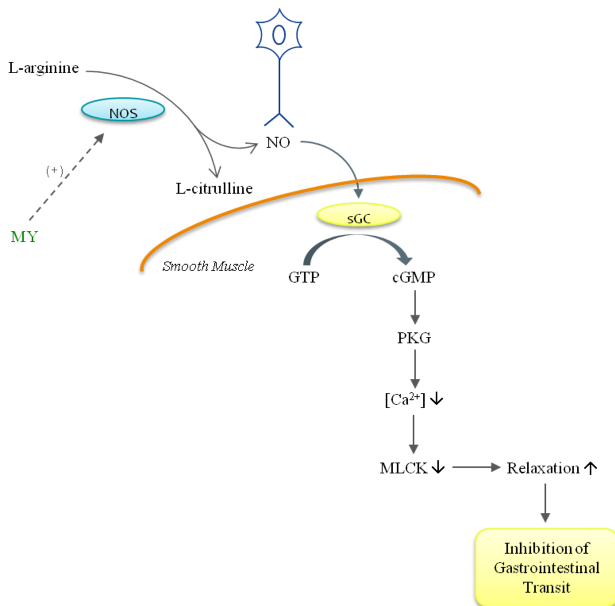

Figure 2: Schematic representation of the probable neurocrine mechanisms involved in the MY induced decrease in the gastrointestinal transit. (+); indicates stimulation, indicates decrease in levels, indicates increase in levels.

The effect of MY on gastrointestinal transit in rats pretreated with L-NAME (nitric oxide synthase inhibitor) and Methylene blue (soluble guanylyl cyclase blocker) has also been studied in order to further investigate the likely neurocrine mechanism responsible for the decreased gastrointestinal transit as a result of suppressed small intestinal motility. In this investigation, intraperitoneal administration of L-NAME (10 mg/kgbw) and MB (1 mg/kgbw) was carried out prior to the application of the test chemical MY. The findings indicate that, in comparison to the effect of MY alone, there has been a significant decrease in the degree of gastrointestinal transit reduction in the L-NAME and MB pre-treated conditions (Figure 1). It could be assumed that the nitrergic antagonists have counteracted the decreased gastrointestinal transit, indicating that the activation/augmentation of nitregic myenteric efferents is responsible for the suppression of small intestinal motility, which in turn causes delayed gastrointestinal transit. MY relaxes the smooth muscles located at the small intestine by means of the soluble guanylyl cyclase signaling pathway mediated by nitric oxide (Figure 2).

CONCLUSION

By inhibiting the contractions of the smooth muscle found in the small intestine's muscularis externa layer, MY delays the gastrointestinal transit by suppressing the contractile activity of the SiVSM. Moreover, the activation of intrinsic nitrergic myenteric efferents, is the cause of the MY-induced suppression of the contractile activity of the SiVSM, which encourages SiVSM relaxation and delays gastrointestinal transit.

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