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Malachite Green Delays the Gastrointestinal Transit in Male Albino Rats

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ABSTRACT

The synthetic food coloring agents are extensively used in various foods because it is cheaper and more stable than natural dyes. Despite of being restricted to use in food items or vegetables, Malachite green (MG), a synthetic dye, is still used as a food adulterant and people are often exposed to toxic MG by consuming contaminated food. Although intoxication by MG has been observed in several animal systems, its potential role in altering contractile activity has not been investigated so far. Thus, the aim of this study was to investigate the effect of MG-induced changes on the contractile activity by assessing gastrointestinal transit with the charcoal meal test. We observed a significant decrease in gastrointestinal transit percentages in groups of rats exposed to MG compared to control rats. Furthermore, significant antagonistic effects on the inhibitory effect induced by MG on the gastrointestinal transit were observed in the pretreated L-NAME and methylene blue rats respectively. It can be concluded that the reason for the decrease in gastrointestinal transit caused by MG may be due to inhibition of smooth muscle contractions located in the wall structure of the small intestine, which ensures motility; facilitating the action of nitric oxide intrinsic myenteric efferents that secrete nitric oxide, the primary inhibitory neurotransmitter.

Keywords: Malachite green, contractile activity, charcoal meal test, intrinsic myenteric efferents, L-NAME, Methylene Blue

INTRODUCTION

It's widely believed that eating green veggies is good for our health. The market is overflowing with an enormous assortment of green vegetables, but buyers sometimes choose to purchase those that look more vibrant and fresher, not realizing that the color they see may not be natural but rather an artificial hue that has been added to make the vegetable appear greener. These synthetic hues may contain dangerous chemicals or dyes that have negative health effects. In addition to green vegetables, ready-to-eat food products with a greenish look also contain artificial green coloring. A variety of substances are utilized as coloring agents such as aniline green and malachite green.

A well-known recalcitrant toxicant, malachite green is a triarylmethane dye with an intricate molecular structure and aromatic rings. Since MG exhibit carcinogenicity, mutagenicity, and teratogenicity (Sudova et al., 2007) it is used to treat external fungal and protozoan diseases in fish. However, it has other applications as an anthelminthic, food additive, colorant, and medicinal disinfectant and as well as a dye used in the industries of silk, wool, jute, leather, cotton, paper, and acrylic (Culp and Beland, 1996). While it's banned globally for usage in food

products, green veggies such as beans, gourds, okra, and peas have supposedly been infused with malachite green to provide the appearance greener and more attractive. In ready-to-eat foods including ice candies, chili sauce, baked goods, and Indian sweets, it was also discovered to be an adulterant. Despite being prohibited in a number of nations and lacking approval from the US Food and Drug Administration (Chang et al., 2001), this dye is nevertheless widely used because of its affordability, accessibility, and effectiveness. Cancer, mutations, and developmental abnormalities can result from prolonged exposure to MG (Srivastava et al., 2004). Malachite green has been described as a mutagen and to cause significant irritation to test animals' eyes and mucous membranes (Mittelstaedt et al., 2004) in a number of toxicological investigations on the dye. Abnormalities in important organs and cancer may result from regular consumption of malachite green.

Humans are exposed to MG in daily life due to consumption of MG contaminated food stuffs and thus are at a high risk of MG intoxication. Any exposure to environmental agents through oral consumption is principally and primarily exposed to the gastrointestinal system and thus assessment of MG induced gastrointestinal toxicity is highly necessary. The gastrointestinal system performs its digestive and absorptive functions through its inherent motor activity which is exhibited as a result of contractions of the visceral smooth muscle cells located in the muscularis externa layer of the intestine. Any impairment in the contractions of the visceral smooth muscle as a result of MG induced intoxication will certainly alter the motor activity of the small intestine and will lead to impaired digestion and malabsorption. The gastrointestinal transit is the *in vivo* method of assessing the gastrointestinal motility. The small intestinal transit has two types of movements i.e. segmentation contraction, where a segment contracts at both ends with bowel, then the content (chyme) is forcefully moved backward and forward until the nutrients are absorbed and peristalsis that initiates the stretch which moves the chyme forward through small intestine. The undigested food finally moves to the large intestine, where the body absorbs water. If transit time alters as a result of MG induced intoxication, it acts as clear indication in assessing any impairment in the motility (motor activity) of the small intestinal visceral smooth muscle. Since no study has been conducted on the effect of MG on the motor activity of SiVSM, the present study has been performed to elucidate the effect of MG on the gastrointestinal transit in order to examine its effect on the motor activity of the SiVSM.

MATERIALS & METHODS

Chemicals

The study utilized reagents and chemicals that were all of analytical grade. The chemicals used in the study include Malachite green, Methylene blue, gum acacia, charcoal 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

Adult female 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.

Table 1. Experimental Setup for the study

Groups	Exposure conditions
Group 1	Application of 40 µM MG orally
Group 2	Application of 60 µM MG orally
Group 3	Application of 80 µM MG 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 80 µM MG orally in L-NAME (10 mg/kg BW) intraperitoneally pre-treated condition
Group 7	Application of 80 µM MG orally 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 ileocaecal 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:

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

Statistical Analysis

The values were represented as the mean ± SEM. Difference among the mean values in groups of each drug treatment and the control was inspected by one way ANOVA in GraphPad Prism 8 software. $P < 0.05$ was considered as significant.

RESULTS AND DISCUSSION

In order to examine the MG induced gastrointestinal toxicity, we have examined the effect of MG on the gastrointestinal transit as an index to assess gastrointestinal motility *in vivo*. From the charcoal meal test, we have observed that MG on oral application significantly decreased the gastrointestinal transit in a dose response manner which is expressed as the percent change in the gastrointestinal transit. As we know the motility of the small intestine helps in propulsion of the food towards anus through its peristaltic movement, so from the results, it is evident that MG induced decrease in the gastrointestinal transit is due to the inhibition of the small intestinal motility caused as result of decreased contraction of the visceral smooth muscle

found in the muscularis externa of the small intestine in response to MG induced intoxication of the small intestinal visceral smooth muscle (SiVSM). It might be hypothesised that the MG induced suppressed motility that led to delayed gastrointestinal transit, which is due to the inactivation of excitatory cholinergic myenteric efferents that secrete acetylcholine (ACh) and/or augmentation of the activity of inhibitory adrenergic myenteric efferents releasing epinephrine or augmentation of the activity of inhibitory nitrergic (NANC, non-adrenergic non cholinergic) myenteric efferents that release Nitric Oxide (NO), the principal neurotransmitter responsible for smooth muscle relaxation.

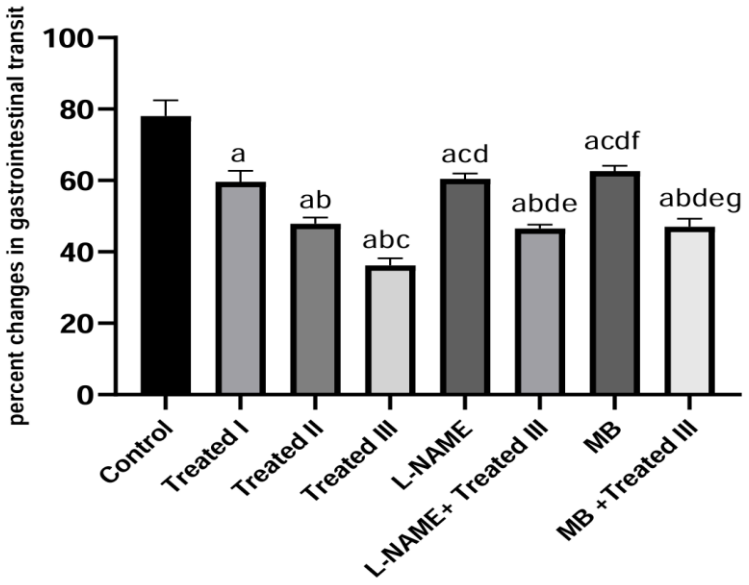

Figure 1. Bar diagram showing percent changes in gastrointestinal transit as a result of the MG induced effects on the contractions of thesmall intestine. The data represented were mean ± SEM for all the group. [a]*P*<0.0001 Vs Control; [b]*P*< 0.0001Vs Treated I; [c]*P*<0.0001 Vs Treated II; [d]*P*<0.0001 Vs Treated III; [e]*P*<0.0001 Vs L-NAME; [f]*P*<0.0001 Vs L-NAME+Treated III; [g]*P*<0.0001 Vs MB.

Further, to investigate the probable neurocrine mechanism involved in the decreased gastrointestinal transit as result of suppressed small intestinal motility, the effect of MG on the gastrointestinal transit in L-NAME (nitric oxide synthase inhibitor) and Methylene blue (soluble guanylyl cyclase blocker) pre-treated rats have been examined. In this study, prior to the application of the test chemical MG, L-NAME (10 mg/kgbw) and MB (1 mg/kgbw) were administered intraperitoneally respectively. From the results, we have found that the degree of decrease in the gastrointestinal transit has been decreased in L-NAME and MB pre-treated conditions in a significant manner as compared to the effect exerted by MG alone. It might be assumed that the nitrergic antagonists have counteracted the decreased gastrointestinal transit suggesting that the suppression of the small intestinal motility that led to delayed gastrointestinal transit is due to the activation/augmentation of nitregic myenteric efferents that induces relaxation of the smooth muscles situated at the small intestine through nitric oxide mediated soluble guanylyl cyclase signaling pathway.

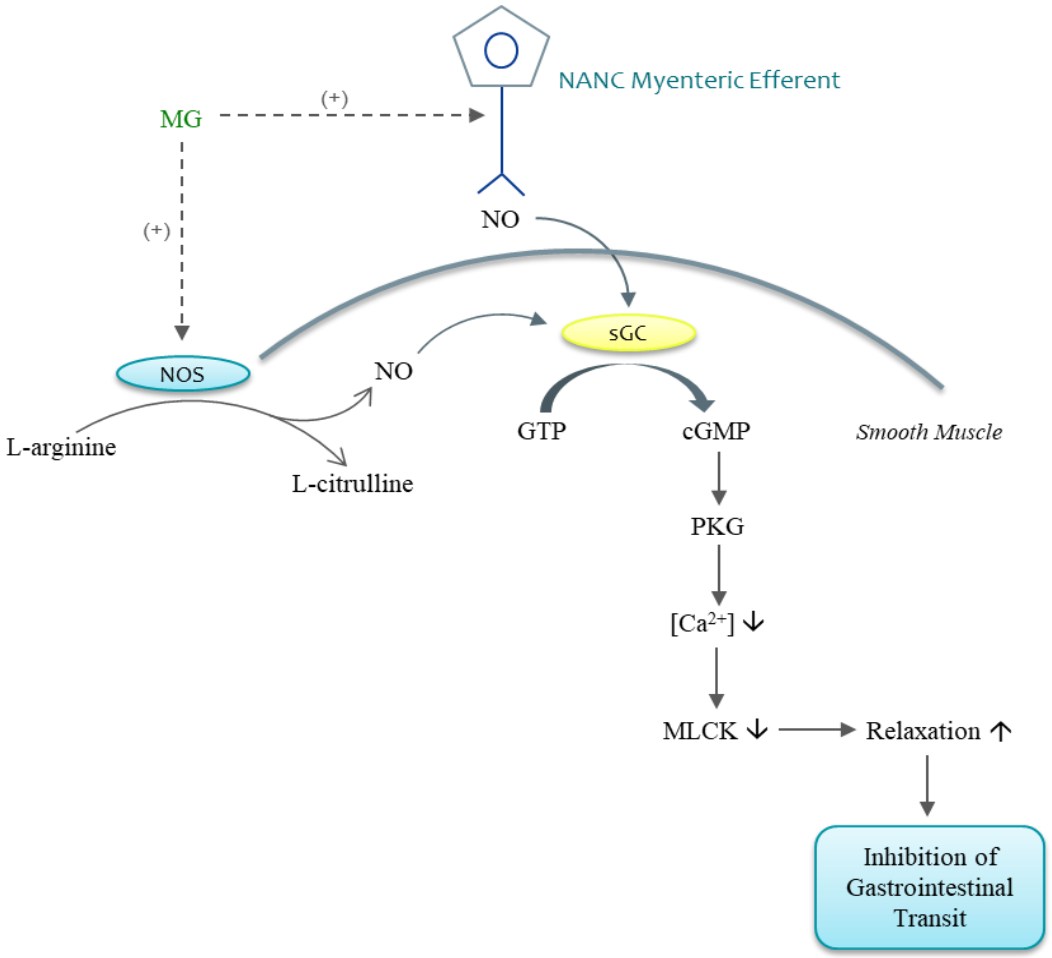

Figure 2. Schematic representation of the probable neurocrine mechanisms involved in the MG induced decrease in the gastrointestinal transit. (+); indicates stimulation, ↓ indicates decrease in levels, ↑indicates increase in levels.

CONCLUSION

MG 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 MG 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.

Declaration by Authors

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Conflict of Interest: The authors declare no conflict of interest.

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