International Journal of Science and Healthcare Research
[Indexed, Peer-Reviewed Journal]
Vol. 10; Issue: 4; Oct.-Dec. 2025
Website: ijshr.com
Original Research Article *ISSN: 2455-7587*

Auramine-O Enhances Small Intestinal Transit in Male Albino Rats

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DOI: https://doi.org/10.52403/ijshr.20250405

ABSTRACT

Auramine O (AO), a synthetic diarylmethane dye, is widely used in various industrial applications but is increasingly scrutinized for its potential toxicological effects on biological systems. Therefore, the present study aimed to investigate the effects of AO on the gastrointestinal transit in male albino rats. Using a charcoal meal test to assess motility of the small intestine, we evaluated the effects of AO on the contractions of the visceral smooth muscles situated at the wall structure of the small intestine. The percentage of intestinal transit was calculated and compared among the groups. We observed a significant increase in gastrointestinal transit percentages in groups of rats exposed to AO compared to control rats suggesting enhanced small intestinal motility. This result suggest that AO facilitates the contraction of the small intestinal smooth muscles thus increasing small intestinal motility. This AO induced facilitation of the contraction of the SiVSM might be due to cholinergic pathway activation which in turn increases the percent small intestinal transit.

Keywords: Auramine O (AO), gastrointestinal transit, acetylcholine, atropine, cholinergic pathway, motility

INTRODUCTION

Auramine-O (AO) is a synthetic diarylmethane dye used in labs as a fluorescent stain (e.g., for acid-fast bacteria) (McCarter & Robinson, 1994) and widely in industries like textiles, paper, and cosmetics. Despite its industrial uses, AO is classified as a potential carcinogen, raising major health concerns due to its unregulated use in food and cosmetic products, especially in developing countries (EFSA, 2015). People are primarily exposed through eating contaminated foods like turmeric powder, sweets, and pickled items from informal markets. Exposure also occurs via skin contact and inhalation in industrial settings (Chung, 2016). Once absorbed, AO is metabolized in the liver into reactive compounds that can cause DNA damage, oxidative stress, and cell death. Animal studies confirm that AO accumulates in organs like the liver, kidneys, and lungs, and chronic exposure has been linked to hepatotoxicity, neurotoxicity, and various cancers (Parodi et al., 1982; IARC, 1987). Human data, though limited, suggests that illegal use of AO in food is associated with

gastrointestinal problems and potential liver complications, with suspicions of a link to gastric and liver cancers.

Synthetic dyes and environmental contaminants, such as AO, are capable of crossing biological membranes and may interact with neurotransmitter systems. Given that AO is a lipophilic compound capable of reaching enteric tissues via systemic absorption, there is a strong rationale to investigate its potential interaction with the enteric nervous system, particularly as it pertains to intestinal motility.

Small intestinal motility is a finely coordinated physiological process responsible for the propulsion of luminal contents through the digestive tract. It is controlled by a complex interplay between the enteric nervous system (ENS), smooth muscle activity, and various neurotransmitters and modulatory substances. The small intestine gets primarily exposed to various environmental agents through oral exposure. Considering the toxicity of AO, it might be expected that AO might alter the contractile pattern of the small intestine exerting its toxicity which might result in altered small intestinal transit impaired digestive and absorptive functions of the small intestine.

Therefore, the present study aims to investigate the effects of Auramine O on small intestinal transit in male albino rats, with an emphasis on deciphering its effect on the contraction of the small intestinal visceral smooth muscle. This approach seeks to provide insights into the potential bioactivity of AO on the gastrointestinal tract beyond its known toxicological profile.

MATERIALS & METHODS

Chemicals and Reagents

All chemicals and reagents used in this study were of analytical grade. Auramine O (AO), the primary test compound, was procured from Sigma-Aldrich. Additional chemicals, including, charcoal, acetylcholine chloride (ACh), atropine sulfate (a muscarinic receptor antagonist) were procured from E. Merck, India.

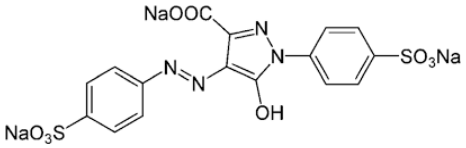

Figure 1: Chemical structure of Auramine O

Experimental Animals

Adult male Sprague Dawley albino rats, aged approximately two to three months and weighing between 130–150 g, were selected as the experimental model. The animals were housed in the departmental animal care facility under standard conditions, maintaining a temperature of 25–27°C with a 24-hour light-dark cycle. They were provided with laboratory chow and water ad libitum. All procedures were conducted in accordance with the guidelines approved by the Animal Ethics Committee of the 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	Control: received distilled water
Set 2	Treated-I: received 20 µM AO
Set 3	Treated-II: received 40 µM AO
Set 4	Treated-III: received 60 µM AO
Set 5	Treated-IV: received 80 µM AO

Sacrifice of the Animals

The selected animals were subjected to overnight fasting prior to sacrifice. Euthanasia was performed via cervical dislocation in strict accordance with the guidelines of the Animal Ethics Committee of the University of Kalyani, ensuring minimal pain and distress to the animals.

Charcoal Meal Test

The animals were fasted overnight prior to the experiment. Following the administration of the test compound via an oral feeding needle, each rat received 0.5

mL of a charcoal meal suspension (10% w/v wood charcoal in 5% w/v gum acacia aqueous solution). After 20 minutes, the animals were euthanized by cervical dislocation. The abdominal cavity was then carefully opened to identify the leading edge of the charcoal marker. To halt peristalsis, the leading edge of the intestine was ligated using cotton thread, or alternatively, the entire small intestine—from the pyloric end of the stomach to the ileocecal junction—was immediately immersed in 5% formalin. The total length of the small intestine and the distance travelled by the charcoal marker were measured. The intestinal segment was gently laid on blotting paper for measurement, taking care to avoid any physical damage to the tissue. The distance travelled by the charcoal meal was recorded and expressed as a percentage of the total intestinal length to calculate the gastrointestinal transit (GIT) percentage using the following 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 effects of Auramine O (AO), we assessed its influence on gastrointestinal transit as a functional index of *in vivo* small intestinal motility using the charcoal meal test. Our results revealed that oral administration of AO significantly increased gastrointestinal transit in a dose-dependent manner, as indicated by the percentage change in charcoal progression through the intestine (Figure 2). Since small intestinal motility is essential for the propulsion of ingested material via peristaltic contractions, the observed acceleration in transit suggests that AO enhances intestinal motor activity.

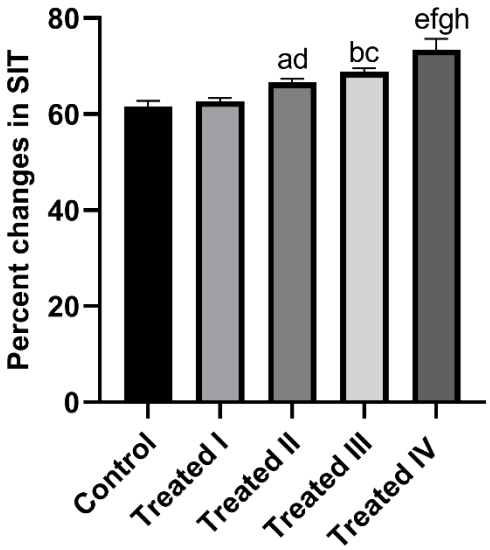

Figure 2. Bar diagram showing percent changes in small intestinal transit (SIT) as a result of the AO induced potentiation of the contractions of the small intestine. The data represented were mean ± SEM for all the group. $^{a,b,c}p<0.01$, 0.001, 0.0001 vs. Control, $^{d,e,f}p< 0.05$, 0.001, 0.0001 vs. Treated I, $^{g}p< 0.001$, vs. Treated II, and $^{h}p<0.01$ vs. Treated III.

This enhanced motility is likely due to increased contractions of the visceral smooth muscle (VSM) located in the muscularis externa of the small intestine, probably resulting from AO-induced stimulation of cholinergic myenteric excitatory neurons within the enteric nervous system and/or inhibition of inhibitory nitrergic and/or adrenergic myenteric efferents (Figure 3).

These findings suggest that Auramine O facilitates the contractions of the visceral smooth muscles located in the small intestinal wall that promotes the motility as evidenced from the increased small intestinal transit. The AO induced impaired motility will hamper the digestive and absorptive functions of the small intestine, and thus rising to several pathological conditions.

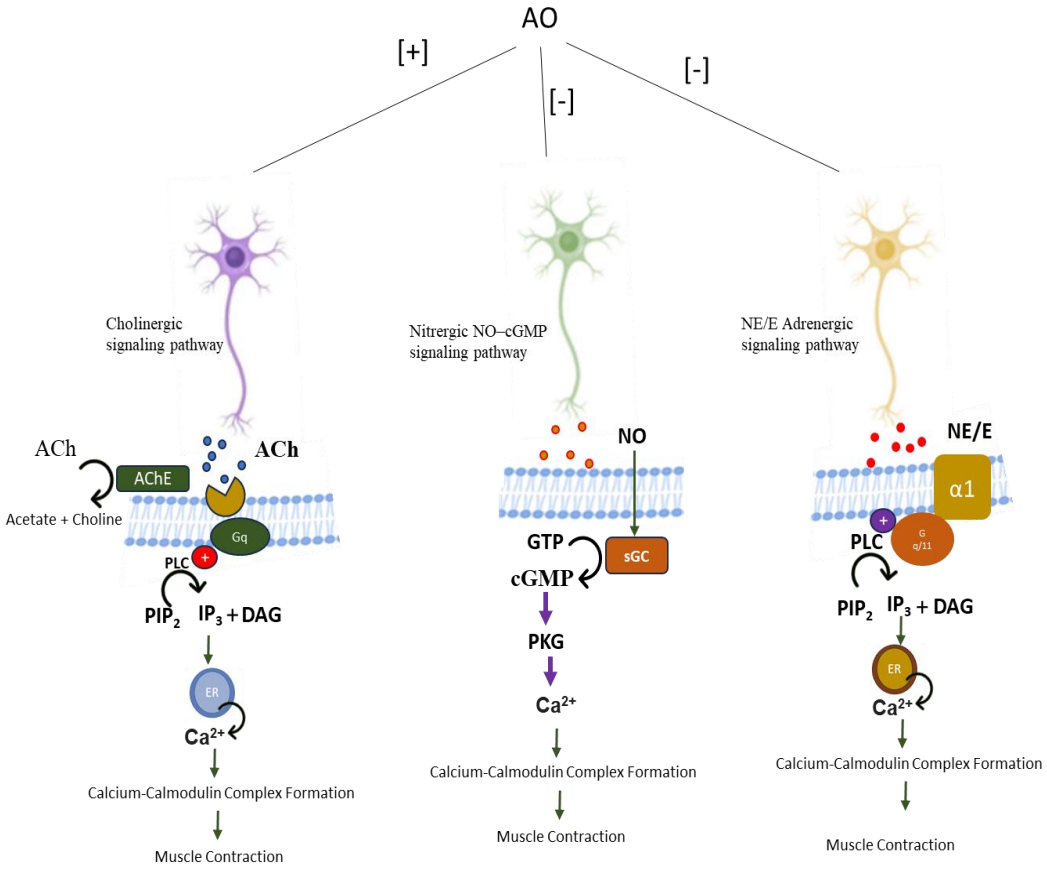

Figure 3. Schematic representation showing the probable neurocrine mechanisms involved in the AO induced facilitation of the contraction of the SiVSM that results in increased small intestinal transit. AO-Auramine-O; ACh-acetylcholine; NO-Nitric Oxide; NE/E- Norepinephrine/Epinephrine; AChE-Acetylcholinesterase; sGC- soluble guanylyl cyclase; [Ca^{2+}]-Intracellular calcium concentration; Cal-Calmodulin; PIP_2 - Phosphatidylinositol 4,5-bisphosphate; IP_3- inositol 1,4,5-trisphosphate; cGMP-cyclic guanosine monophosphate; MLCK- Myosin light chain kinase. +, indicates facilitation; - , indicates inhibition.

CONCLUSION

The findings of the present study clearly indicate that Auramine O (AO) significantly enhances small intestinal transit *in vivo*, reflecting a stimulatory effect on small intestinal motility. This prokinetic action appears to be dose-dependent and is likely mediated through the increased contractility of the small intestinal visceral smooth muscle (SiVSM) located within the muscularis externa. The observed enhancement in motility is strongly suggestive that the facilitation of the activity of cholinergic myenteric efferents and/or suppression of the activity of nitrergic/adrenergic myenteric efferents

might be responsible for it. Collectively, the results suggest that the AO-induced facilitation of gut motility is neurochemically driven and also raise important concerns about the potential gastrointestinal risks associated with chronic exposure to Auramine O, emphasizing the need for further toxicological and mechanistic studies.

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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How to cite this article: Rimi Patra, Sandhi Paul, Raina Ghosh, Sourapriya Mukherjee, Kamalesh Das, Goutam Paul. Auramine-O enhances small intestinal transit in male albino rats. *Int. J. Sci. Healthc. Res.* 2025; 10(4): 28-32. DOI: *https://doi.org/10.52403/ijshr.20250405*

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