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Malachite Green Depresses The Contractile Activity Of The Duodenal Visceral Smooth Muscle By Facilitating The Activity Of Intrinsic Nitrergic Efferents

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Abstract: The small intestine is exposed to Malachite green (MG), a synthetic dye, commonly restricted to use as food colorant mainly through MG colored foods and vegetables. MG possesses toxicity and it is used as insecticides in aquatic system. So, on consumption of MG, it might exert its toxicity and impair the functioning of human health. However, the toxic effect of MG, especially on the motor function of duodenal visceral smooth muscle, has not been studied till date. Thus, the aim of the study was to investigate the potential toxic effects of MG on duodenal visceral smooth muscle (dVSM) contraction ex vivo in rat model. The results showed that duodenal motility was significantly decreased when duodenal segments were exposed to MG in a dose-dependent manner. In addition, we demonstrated a significant counteraction of the inhibition of the contractions of the dVSM induced by MG in L-NAME and Methylene Blue pre-treated duodenal preparations in order to examine the involvement of inhibitory nitrergic myenteric efferents in the MG induced contractile activity of the dVSM. Therefore, it can be suggested that MG inhibits the contractions smooth muscles of the duodenal viscera, probably by increasing the activity of intrinsic myenteric nitrergic efferents through NO-mediated sGC signaling pathway.

Keywords - Malachite green, duodenal visceral smooth muscle, nitrergic myenteric efferents, L-NAME, MB

I. INTRODUCTION

Many people believe that consuming green vegetables is beneficial for our health. The market has a wide variety of green vegetables, and consumers often opt for those that appear more vibrant and fresher. However, they may not realize that the vivid color is not natural but rather an artificial hue added to make the vegetables look greener. These synthetic colors may contain harmful chemicals or dyes that can have adverse effects on health. Apart from green vegetables, processed food items with a greenish appearance also contain artificial green coloring. Various substances, including aniline green and malachite green, are used as coloring agents.

Malachite green, a well-known stubbornly resistant toxic substance, is a type of triarylmethane dye characterized by a complex molecular structure and aromatic rings. Due to its reported carcinogenic, mutagenic, and teratogenic effects (Sudova et al., 2007). MG is employed for the treatment of external fungal and protozoan infections in fish. Nevertheless, it also finds uses as an anti-helminthic, food preservative, coloring agent, medicinal disinfectant, as well as a dye in the silk, wool, jute, leather, cotton, paper, and acrylic industries (Culp and Beland, 1996). Although globally banned for use in food products and green vegetables like beans, gourds, okra, and peas have reportedly been treated with MG to enhance their green appearance

and make them more appealing. MG has been found to be an adulterant in ready-to-eat foods such as ice candies, chili sauce, baked goods, and Indian sweets. Despite being prohibited in numerous countries and lacking approval from the US Food and Drug Administration (Chang et al., 2001), this dye is still widely used due to its cost-effectiveness, availability, and efficacy. Prolonged exposure to MG can lead to cancer, mutations, and developmental abnormalities (Srivastava et al., 2004). The dye malachite green has been found to be a mutagen and is known to cause considerable irritation to the eyes and mucous membranes of test animals (Mittelstaedt et al., 2004) in several toxicological studies. Regular consumption of MG may lead to abnormalities in vital organs and could result in cancer.

MG is frequently encountered by humans when they consume foods contaminated with the malachite green. Upon consuming malachite green, it primarily exposed to the small intestine. The small intestine has a crucial role in controlling digestive and absorptive functions through its motor function, which is achieved by contractions of the visceral smooth muscle cells situated in the wall structure of the small intestine. Various motor patterns assist in mixing the luminal contents with succus entericus and other enzymes, aiding in the aboral movement of the intestinal contents for excretion. Assuming the toxicity of MG, it can be expected that the consumption of MG may result in alterations in the motor functions of the small intestine, leading to inadequate digestion and malabsorption. Since the impact of MG on the contractile (motor) function of the small intestine, the organ responsible for digesting and absorbing MG -contaminated food, is not clearly established, the current study aims to clarify the pharmacodynamics of MG -induced effects on the contractile functions of duodenal visceral smooth muscle (dVSM), an integral part of the small intestine in albino rats.

II. MATERIALS AND METHODS

CHEMICALS

The study made use of analytical grade reagents and chemicals exclusively. The experimented chemical utilized- Malachite green,methylene blue (MB), potassium chloride (KCl), sodium chloride (NaCl), magnesium chloride ($MgCl_2$), calcium chloride ($CaCl_2$), sodium bicarbonate ($NaHCO_3$), sodium dihydrogen phosphate (NaH_2PO_4), and glucose, were acquired from E-Merck in India.N-ω-nitro-L-arginine methyl ester hydrochloride (L-NAME) was procured from Sigma Aldrich, USA.

ANIMALS

The experimental setup involved the selection of male albino rats from the Charles Foster strain, with a weight ranging from 130-150 grams. These rats were housed in the departmental animal house at a temperature of approximately 25-27°C and a 24-hour light-dark cycle. They had unrestricted access to laboratory chow and water. All experiments were conducted in compliance with the animal ethics committee's guidelines at Kalyani University.

EXPERIMENTAL SET UP AND EXPOSURE CONDITION

Malachite green was mixed with distilled water. For the current study, four varying concentrations of MG - 20 µM, 40 µM, 60 µM, and 80 µM - were prepared. The animals were exposed to different doses and exposure conditions as mentioned in Table 1.

Table 1. Experimental Setup for the study

Groups	Exposure conditions
Set 1	Application of graded doses of Malachite green (20, 40, 60, and 80 µM) on the duodenal segments
Set 2	Application of single dose of L-NAME (200 µM) on the duodenal segments
Set 3	Application of highest dose of Malachite green (80 µM) on duodenal segments pretreated with L-NAME (200 µM)
Set 4	Application of single dose of MB (200 µM) on the duodenal segments
Set 5	Application of effective dose of Malachite green (80 µM) on duodenal segments pretreated with MB (200 µM)

ANIMAL SACRIFICE

The night before the experiments, the animals were kept on an empty stomach. For painless sacrifice, animals were subjected to cervical dislocation according to the guidelines of Kalyan University Animal Ethics Committee.

COLLECTION OF THE TISSUE

Immediately following the cervical dislocation, the abdominal region of the sacrificed animal was incised to collect the small intestine. The small intestine's duodenal section was then separated from the mesentery through surgical means. The duodenum was chosen for the experiment due to its higher motility compared to other sections. Following the separation, the duodenal's lumen was thoroughly cleaned through a gentle and meticulous flushing process to ensure the complete removal of its contents. To document the duodenal motility, the separated tissue was quickly placed in the Dale's apparatus organ bath, ensuring the process began as soon as the animal was sacrificed.

RECORDING THE DUODENAL MOVEMENT

To document the movement pattern of the duodenal part of the small intestine, which is approximately 3 cm long, a section of the duodenal tissue was removed and positioned upright using two metal hooks that went through the tissue from both ends. This section was then placed in an organ bath containing 45 ml of Tyrode's solution. The Tyrode's solution was prepared by mixing 8.0 g of sodium chloride (NaCl), 0.2 g of potassium chloride (KCl), 0.2 g of calcium chloride ($CaCl_2$), 0.1 g of magnesium chloride ($MgCl_2$), 0.05 g of sodium di-hydrogen phosphate (NaH_2PO_4), 1.0 g of sodium bi-carbonate ($NaHCO_3$), and 1.0 g of dextrose into a total volume of 1L, maintaining a pH of 7.4. The organ bath was equipped to provide a steady supply of oxygen (95% O_2 and 5% CO_2) to the tissue through an oxygen bubbler. The temperature of the isolated tissue was kept at 37±0.5°C using an automatic thermostat machine connected to the Dale's apparatus.

To measure the movement of the vertically isolated tissue segment, its lower end was secured to the bottom of the organ bath with a metal hook, and its upper end was similarly attached to the lever of an isotonic transducer apparatus (IT-2245) connected to RMS Polyrite D (RMS, Chandigarh, India). Each segment was given a minimum of 30 minutes to stabilize under the experimental conditions and was regularly rinsed with

fresh Tyrode's solution to remove any accumulated metabolites. The final step involved recording the isotonic contraction, which was achieved by applying various doses of malachite green and then some blockers.

III. Statistical Analysis

The information on each test group's data was shown as mean ± standard error of mean (SEM). The strength of contractions was calculated by considering the number of cycles and the peak intensity of the movement data. The difference in percentage change from the initial (or baseline) values was used to indicate the outcomes of the experimental treatments during the functional assessments. To assess if there were any notable variances among the groups, a one-way analysis of variance (ANOVA) was conducted using GraphPad Prism 8, with a significance level of $P < 0.05$ being deemed significant.

IV. Results and Discussion

EFFECT OF MALACHITE GREEN ON THE CONTRACTILE ACTIVITIES DUODENAL SMOOTH MUSCLE *EX VIVO* OF RAT

The potency of the duodenal smooth muscle (dVSM) to contract in response to various concentrations of MG was evaluated by observing the duodenum's movements during single-dose acute experiments. Upon examining the tracings, it was found that with increase in the concentration of MG, the amplitude and frequency of the dVSM contractions decreases dose dependently (Figure 1). At the 80 µM concentration, the duodenal contractions were suppressed by over 80%, leading to a complete halt in contractions for the remainder of the test.

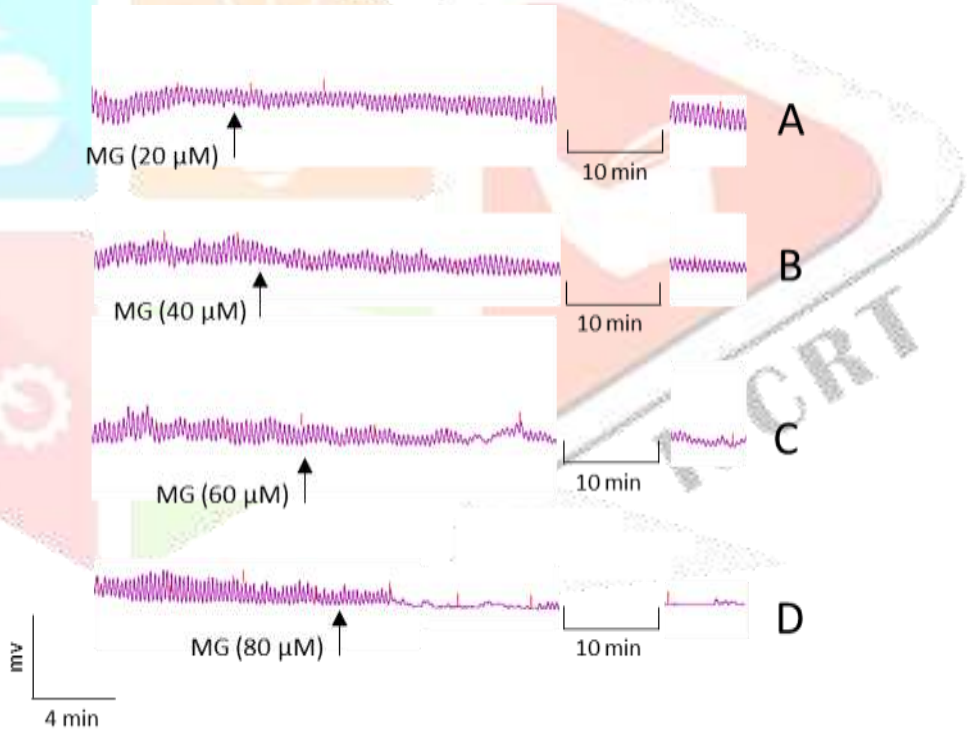

Figure 1. Tracings showing representative records of the effect of graded concentrations of MG on the isolated duodenal movement of rat in tissue organ bath obtained with an isotonic transducer coupled to RMS Polyrite-D.

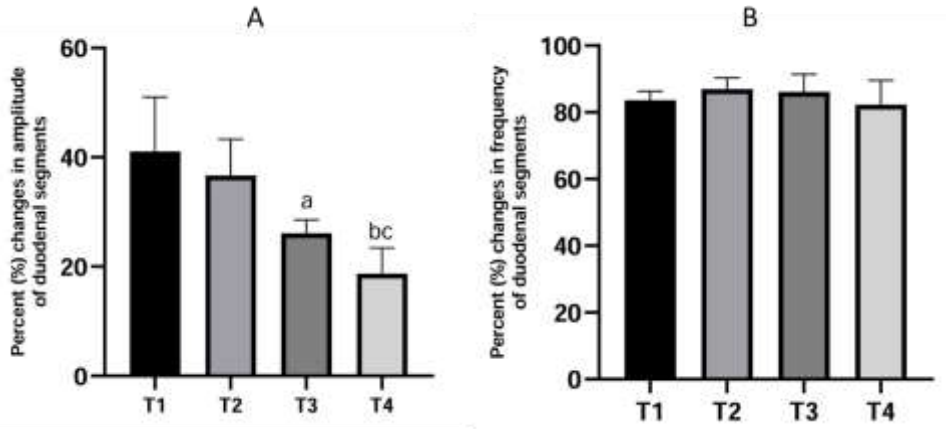

Figure 2. Bar diagram showing percent changes in amplitude and frequency of the contractions of isolated duodenum in response to the application of graded doses of MG. The data represented were mean ± SEM for all the group. $^{a,b}P$<0.05, 0.001 Vs T1; ^{c}P< 0.01Vs T2(A).

These results indicate that MG suppresses the contractile activities of the dVSM by reducing the force (strength) within the smooth muscle of the duodenum's walls. No significant alterations in the frequency of contractions of the dVSM has been observed. The contractions of the visceral smooth muscle in the digestive tract are mainly controlled by the muscle's own intrinsic nerves through actions of both excitatory cholinergic, inhibitory adrenergic, and inhibitory nitrergic (NANC) nerve signals. It is hypothesized that the effect of MG on the dVSM contractions might be due to blocking the action of cholinergic nerve signals or by stimulating the activity of adrenergic/nitrergic (NANC) intrinsic nerves innervating the dVSM.

EFFECT OF MALACHITE GREEN AND NITRERGIC ANTAGONISTS IN COMBINATION ON THE CONTRACTILE ACTIVITIES DUODENAL SMOOTH MUSCLE *EX VIVO* OF RAT

To elucidate the probable pharmacodynamic of MG -induced inhibition on the contractile activities of the dVSM, we examined the role of nitrergic intrinsic myenteric efferents through observing the effect of MG in combination with nitrergic antagonist in single dose experiment study. Nitrergic myenteric efferents releases Nitric Oxide (NO), the principal inhibitory neurotransmitter that inhibit contractions of the visceral smooth muscle in the duodenal wall and promotes relaxation of the visceral smooth muscle.

To understand the involvement of nitrergic activity in MG induced suppression of the dVSM's contraction, we carried out an experiment using a single dose to observe the movement of the duodenum when exposed to MG and L-NAME, a Nitric Oxide Synthase (NOS) inhibitor. The recordings indicated that L-NAME on its own didn't significantly alter the dVSM contractions. However, when MG was introduced together with L-NAME, the degree of inhibition of the dVSM contractions were lessened compared to those experienced with just MG. This outcome clearly indicates that MG enhances the function of nitrergic myenteric efferents by increasing the activity of nitric oxide synthase, the enzyme responsible for the production of Nitric Oxide in these cells.

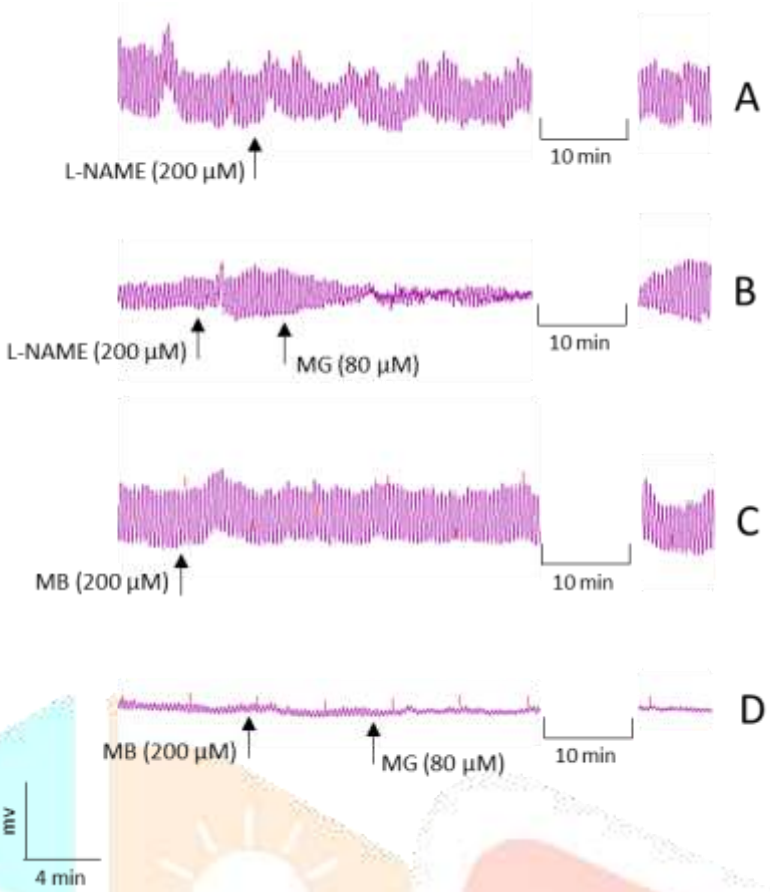

Figure 4. Tracings showing representative records of the effects of malachite green on the movement of duodenum in L-NAME and MB pre-treated duodenal preparations *ex vivo*. A: Tracing of effect of L-NAME (200 µM) on the movement of duodenum. B: Tracing of the effect of MG (80µM) on the movement of duodenum in L-NAME (200 µM) pre-treated duodenal preparations. C:Tracing of the effect of MB (200 µM) on the movement of duodenum. D: Tracing of the effect of MG (80µM) on the movement of duodenum in MB (200 µM) pre-treated duodenal preparations obtained with an isotonic transducer coupled to RMS Polyrite-D.

Further, to investigate the involvement of the nitrergic signaling pathway in the suppression of dVSM contractions caused by MG, the movement of the duodenum was recorded in response to the application of MG and MB, a compound that blocks the nitrergic signaling pathway (sGC blocker). *Ex vivo* observations were made to track the response. The recorded data showed that MB by itself did not significantly alter the contraction of dVSM. Yet, when MG was applied in combination with MB, it counteracted the inhibitory effect of MG on dVSM contraction compared to when Malachite green was applied alone.

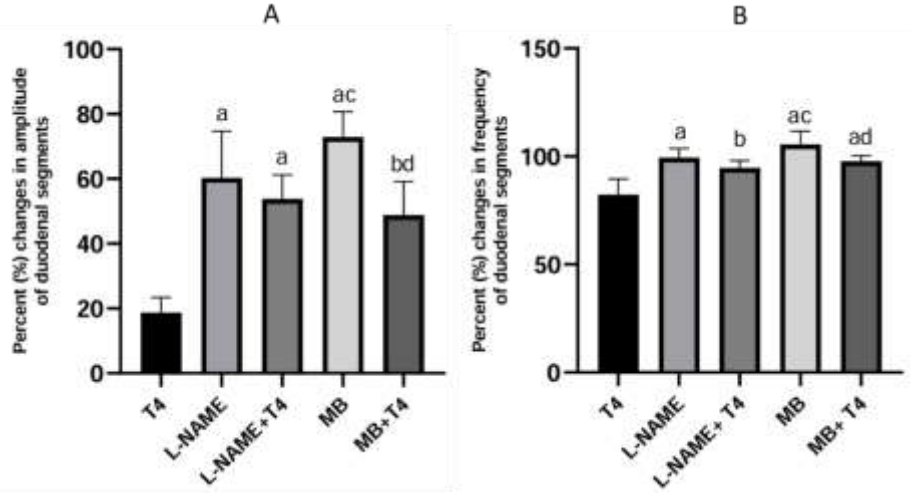

Figure 5. Bar diagram showing percent changes in amplitude and frequency of the contractions of isolated duodenum in response to the application of MG in combination with L-NAME and MB respectively. The

data represented were mean ± SEM for all the group. [a,b]*P*< 0.0001, 0.001 Vs T4; [c]*P*< 0.05 Vs L-NAME+T4; [d]*P*<0.01 Vs MB (A). [b,a]*P*< 0.001, 0.0001 Vs T4; [c]*P*< 0.01 Vs L-NAME+T4; [d]*P*< 0.05 Vs MB (B).

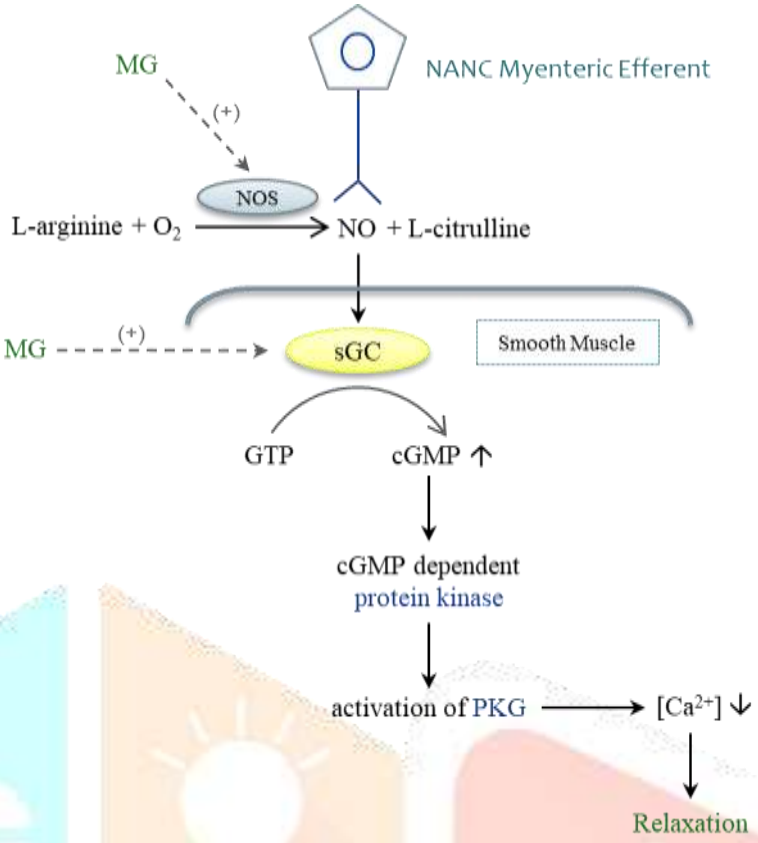

Figure 6.Schematic representation of the probable neurocrine mechanisms involved in the MG induced suppression of the contractile activity of the dVSM. (+); indicates stimulation, (-); indicates inhibition, ↓ indicates decrease in levels, ↑ indicates decrease in levels.

These results suggest that both L-NAME and MB, which act as nitrergic antagonists, counteracted the inhibitory effects of MG on dVSM contraction. This strongly supports that Malachite green reduces dVSM contractions by augmenting the activity of intrinsic nitrergic myenteric efferents through the nitric oxide-activated soluble guanylyl cyclase pathway.

V. CONCLUSION

MG is a synthetic dye often used as a food adulterant. It is concluded that MG inhibits the contractile activity of the dVSM by inhibiting the contractions of the visceral smooth muscle in the duodenal wall that provides the motility. MG suppresses the contractile activity of the dVSM probably by augmenting the soluble guanylyl cyclase mediated nitrergic signaling pathway. The extrapolations from the study reveal that MG on chronic consumption might inhibit the contractile activity of the dVSM resulting in impaired digestion and absorption in humans.

VI. ACKNOWLEDGMENT

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REFERENCES

[1] Cha, C. J., Doerge, D. R., & Cerniglia, C. E. (2001). Biotransformation of malachite green by the fungus Cunninghamella elegans. Applied and environmental microbiology, 67(9), 4358-4360.

[2] Culp, S.J., Beland, F.A., 1996. Malachite green: a toxicological review. J. Am. Coll. Toxicol. 15, 219–238.

[3] Curricular on Food Safety (2003) Directorate General of Health Services, Ministry of Health & Family Welfare, New Delhi, India, pp 70–70

[4] Mittelstaedt, R. A., Mei, N., Webb, P. J., Shaddock, J. G., Dobrovolsky, V. N., McGarrity, L. J., ... & Heflich, R. H. (2004). Genotoxicity of malachite green and leucomalachite green in female Big Blue B6C3F1 mice. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 561(1-2), 127-138.

[5] Srivastava S, Sinha R, Roy D. Toxicological effects of malachite green. Aquatic Toxicology. 2004 Feb 25; 66(3):319-29. https://doi.org/10.1016/j.aquatox.2003.09.008

[6] Sudova, E., Machova, J., Svobodova, Z., & Vesely, T. (2007). Negative effects of malachite green and possibilities of its replacement in the treatment of fish eggs and fish: a review. Veterinarnimedicina, 52(12), 527.