

# Silver Nanoparticles as a Breakthrough Therapy for Irritable Bowel Syndrome

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## INTRODUCTION & AIM

**Irritable bowel syndrome (IBS)** is a multifactorial disorder, presenting with symptoms like altered intestinal motility, visceral hypersensitivity, and dysfunction of the gut-brain axis. The most common therapeutic strategies rely on antispasmodic agents (mebeverine, drotaverine), laxatives, antidiarrheals (loperamide, scopolamine), 5-HT<sub>3</sub> receptor antagonists (alosetron), and anti-inflammatory agents [1-4].

The nanoparticle (NP) system is one of the best options for targeted therapy. It provides the medication in a controlled manner to a specific area of inflammation. Silver nanoparticles (Ag NPs) are already utilized as reliable drug-delivery systems in the pharmaceutical industry. Therefore, the current study aims to **develop drug-loaded Ag NPs with the antispasmodic mebeverine (Meb) on their surface to improve the treatment of IBS** and study the biological effects of this novel dosage form.

## METHOD

### Ag NPs synthesis

Ag NPs are prepared by the consecutive mixing of 1.25 g glucose, 25 mL H<sub>2</sub>O, and 0.63 mL 0,01 M AgNO<sub>3</sub>. The mixture is then stirred and refluxed for 5 minutes [5]. Mebeverine-loaded Ag NPs (Meb-Ag NPs) are obtained according to the same procedure, but when the reaction mixture reached 100°C, 1 mg Meb was added.

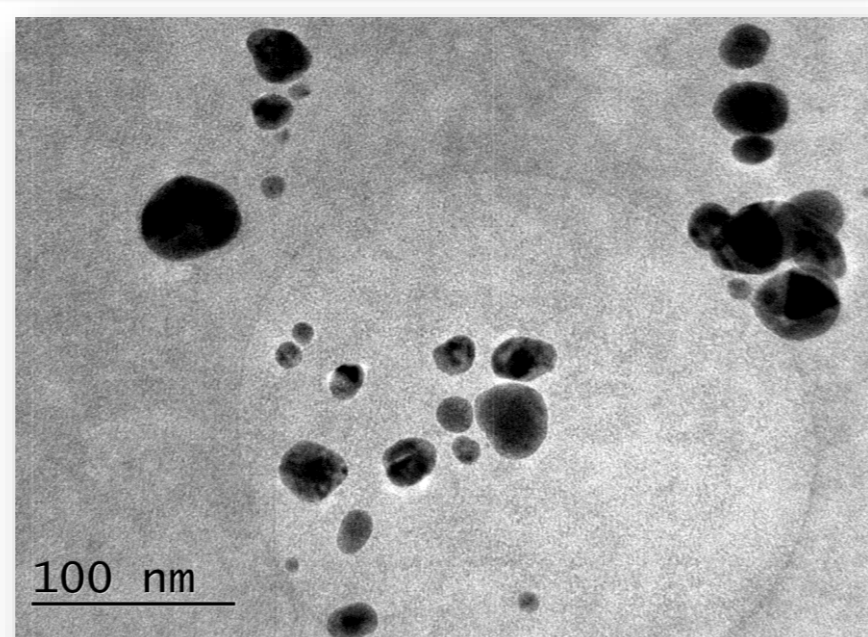
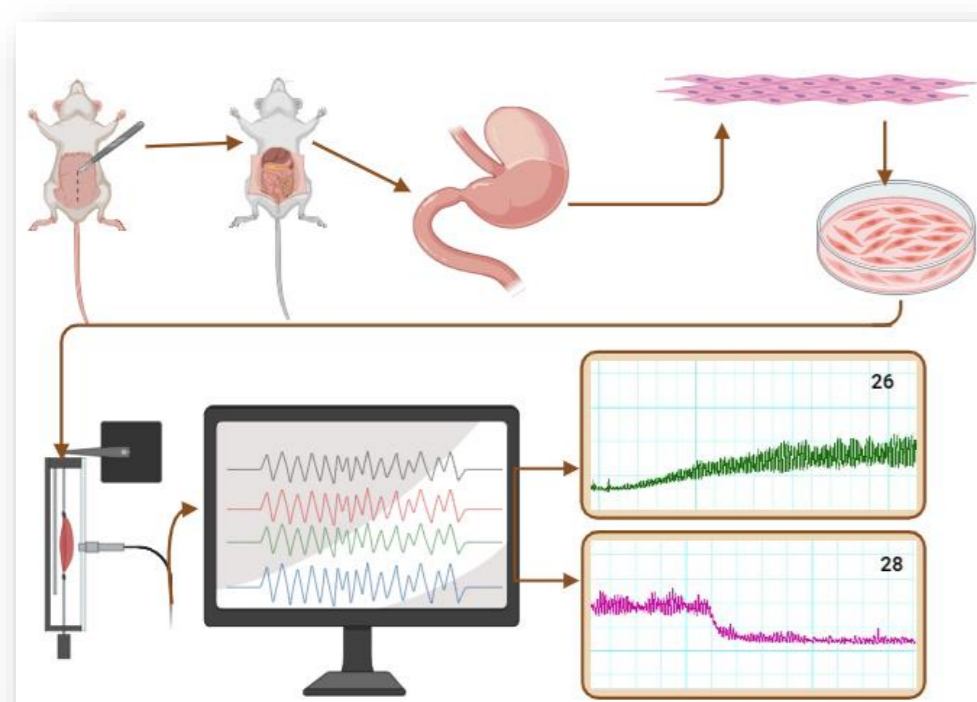


Figure 1. TEM image of Meb-Ag NPs.

### Spasmolytic activity assessment

The effect on spontaneous smooth muscle (SM) activity was studied through the isolated organ bath method. Circular SM preparations were obtained from the gastric corpus of male Wistar rats. Tested solutions of Ag NPs are added to the tissue baths, then the effect of each substance is registered and recorded graphically.



### Ex vivo anti-inflammatory activity assessment

Immunohistochemical staining was performed on formalin-fixed, paraffin-embedded 4 μm sections after retrieval of antigenic epitopes with citrate pH 6.0, endogenous biotin, and peroxidase block, using an Autostainer Link 48. Images were visualized and captured on a Nikon Eclipse 80i microscope.



## FUTURE WORK / REFERENCES

Building on these promising outcomes, future research will aim to investigate their cytotoxicity, genotoxicity, and DNA damage activity. Such findings would bring more insight into the potential of drug-loaded Ag NPs as a drug-delivery system for various diseases.

- [1] Hicks, G.A. 6.29—*Irritable Bowel Syndrome*; Taylor, J.B., Triggler, D.J., Eds.; Comprehensive Medicinal Chemistry II; Elsevier: Amsterdam, The Netherlands, 2007; [2] Milusheva, M. et al. *Pharmaceuticals* 2023, 16, 1660. [3] Milusheva, M. et al. *Molecules* 2024, 29, 3375. [4] Stoyanova, M. et al. *Biomedicines* 2024, 12, 2321. [5] Todorova, M. et al. *Biomedicines* 2023, 11, 1593.

## RESULTS & DISCUSSION

Meb-Ag NPs were characterized by recording their UV-visible spectra, zeta potentials, DLS, and TEM (Fig. 1). Zeta potential value of **-6.12 mV** was determined, which confirms glucose oxidation to gluconic acid.

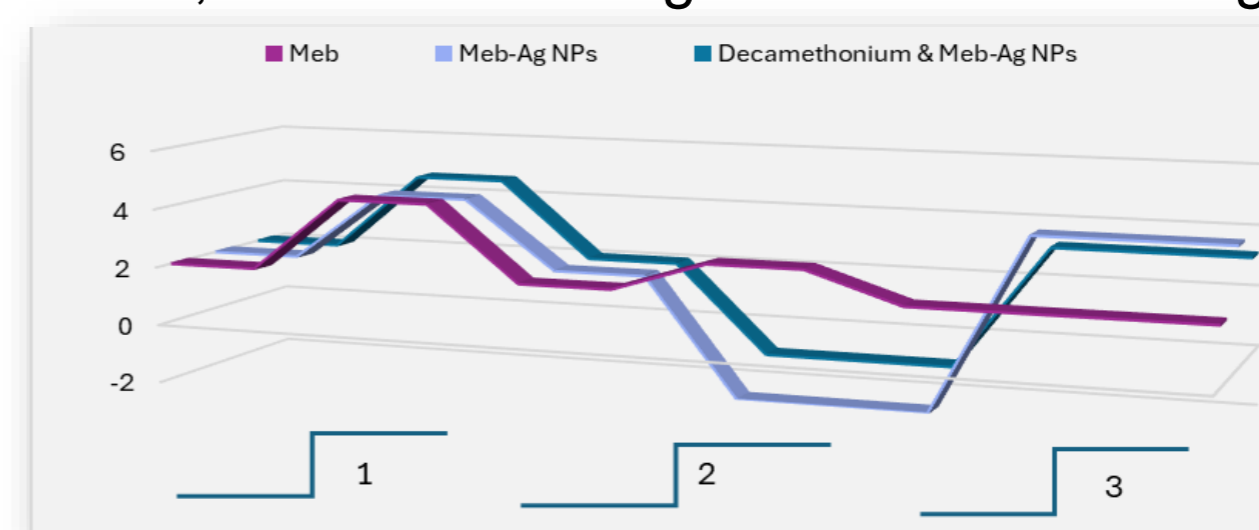


Figure 2. SM responses to ACh (Sections 1 and 3), and the effects of Meb, Meb-Ag NPs, and decamethonium combined with Meb-Ag NPs (Section 2).

A weak relaxation response of SM preparations is observed when treated with Meb (Fig. 2). Meb causes a complete absence of the tonic component (100% reduction) in the acetylcholine (ACh) reaction. Most likely, muscarinic (mAChR) and nicotinic (nAChR) cholinergic receptors are influenced. **In contrast, the Meb-Ag NPs showed relaxation effect.** Decamethonium, a partial nAChR-agonist and depolarizing microrelaxant, was used for pre-treatment. The SM response induced by Meb-Ag NPs remained unchanged following this pre-treatment, suggesting that the **pharmacological response mediated by the Meb-Ag NPs does involve the cholinergic receptor pathway.**

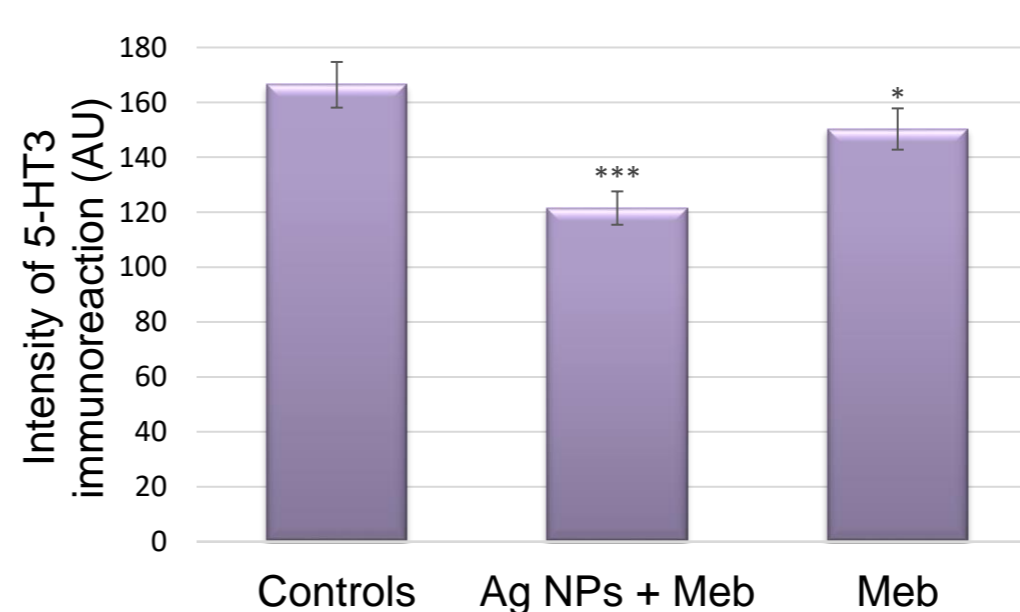


Figure 3. Intensity of IL-1β and 5-HT<sub>3</sub> immunoreaction (in arbitrary units) in rat stomach smooth muscle preparations. \*\*\*,  $p < 0.001$ ; \*,  $p < 0.05$ .

It is known that both IBS and Crohn's disease stimulate the production of inflammatory factors by immune cells. We found that Meb-Ag NPs showed **greater anti-inflammatory activity** than the control samples **regarding IL-1β expression.** They also demonstrated **higher inhibitory activity** at the 5-HT<sub>3</sub> channel receptor compared to Meb and controls (Fig. 3).

## CONCLUSION

The application of new drugs obtained through nanotechnology for the treatment of IBS holds great promise for local delivery and reduction of side effects. With that aim, a **green, glucose-assisted method was used to synthesize Meb-Ag NPs.** Characterization of the synthesized Ag NPs, including their shape, size distribution, and surface charge, was performed using different spectroscopic techniques.

*Ex vivo* experiments on rat gastric smooth muscles **revealed a direct relationship between the activation of mAChR and nAChR**, which we consider to be the underlying cause of the biological activity of Ag NPs. Meb-Ag NPs showed **better anti-inflammatory activity** than the control samples regarding IL-1β expression and 5-HT<sub>3</sub> receptors.

These results indicate that **Ag NPs have the potential of an effective drug delivery system for Meb in IBS treatment**, possibly benefiting millions of patients globally.

## ETHIC STATEMENT AND ACKNOWLEDGEMENTS

All procedures were conducted in full compliance with EU and Bulgarian national regulations, as well as the university's internal ethical standards.

**The results are part of Scientific Project KP-06-H73/11 of the National Fund for Scientific Research in Bulgaria, National Program for Basic Research Projects 2023.**