

# Antidiabetic Activity and Cytotoxic Effect of Selenium Nanoparticles Mediated Through Mimosa Pudica Leaves Extract

Dhave Prasath V M<sup>1</sup>, Aswin Sundar R<sup>2</sup>, Rajeshkumar Shanmugam<sup>1\*</sup>,  
Lakshmi Thangavelu<sup>3</sup>, Dhanyaa Muthukumaran<sup>1</sup>

<sup>1</sup>Nanobiomedicine Lab, Centre for Global Health Research, Saveetha Medical College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Chennai, India

<sup>2</sup>Department of Orthopaedics, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences, Chennai, India

<sup>3</sup>Centre for Global Health Research, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences, Chennai - 602105, Tamil Nadu, India  
Email: [rajeshkumars.smc@saveetha.com](mailto:rajeshkumars.smc@saveetha.com)

The antidiabetic and Cytotoxic effects of Se NPs synthesized by using Mimosa pudica (M.pudica) were examined in this study. The study aims to green synthesis of M. pudica-mediated Selenium Nanoparticles(Se NPs) and elucidate the antidiabetic activity of the synthesized M. pudica-mediated Se NPs. Selenium exhibits significant antidiabetic properties through its antioxidant, anti-inflammatory, and insulin-sensitizing effect The study evaluates brine shrimp lethality assay, alpha-amylase and beta-glucosidase of M. pudica - Se NPs. Both assays verified that M. pudica-mediated Se NPs effectively inhibited the activity of  $\alpha$ -amylase and  $\beta$ -glucosidase in a dose-dependent manner. The Se NPs showed substantial inhibitory effects, although slightly lower than the standard antidiabetic drug. low cytotoxic effect on Brine Shrimp Lethality Assay (BSLA), with high survival rates maintained even at elevated concentrations. The slight decrease in survival rates on the second day at higher concentrations suggests a dose-dependent response, but overall, the Se NPs were well-tolerated by the nauplii within the tested concentration range.

**Keywords:** Antidiabetic activity, Cytotoxicity Assay, Green Synthesis, Mimosa pudica, Selenium Nanoparticles.

## 1. Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by high blood glucose levels resulting from defects in insulin secretion, insulin action, or both. It is a major global health concern, affecting approximately 463 million adults worldwide (Bashary et al., 2020). The

complications associated with diabetes, such as cardiovascular disease, kidney disease, and blindness, can be debilitating and life-threatening. The prevalence of diabetes is expected to rise to 578 million by 2030, highlighting the need for effective prevention and management strategies. (El-Badri et al., 2022)

Selenium is an essential micronutrient that plays a vital role in many physiological processes, including antioxidant defence, and immune function. In recent years, selenium nanoparticles (Se NPs) have emerged as a promising area of research due to their unique physicochemical properties and potential therapeutic applications (Jayapriya et al., 2022, Johnson et al., 2024, Shanmugam et al., 2023, Kathiravana et al., 2023). In the therapeutic field, it is used in oxidoreductase activity. (Huang et al., 2023) (Song et al., 2023). Se NPs exhibit high surface area-to-volume ratios, enhanced reactivity, and increased bioavailability compared to bulk selenium. They have been shown to possess strong antioxidant and anti-inflammatory activities, as well as antitumor, antimicrobial, and antiviral properties. (L'Heveder & Nolan, 2013) (Khurana et al., 2023).

Furthermore, Se NPs have been investigated for their potential use in drug delivery systems, due to their ability to encapsulate and release therapeutic agents in a controlled manner (Khurana et al., 2019). They have also been studied for their potential application in food and agriculture (Lovic et al., 2020). Despite their potential benefits, the safety and toxicity of Se NPs are still under investigation, and the mechanisms of their biological activities are not fully understood. Nevertheless, the development of Se NPs as a new class of nanomaterials holds great promise for a wide range of biomedical and industrial applications (Malayan et al., 2013).

*M. pudica*, also known as the sensitive plant, has been traditionally used in Ayurvedic medicine for its medicinal properties (Muhammad et al., 2015) (Othman et al., 2022). Several studies have reported the antidiabetic activity of *M. pudica* extracts in animal models and in vitro studies. The study found that the extract significantly inhibited  $\alpha$ -amylase and Beta glucosidase, which are involved in carbohydrate digestion, indicating its potential as an antidiabetic agent. (Elechi & Unamba, 2023) (Piacenza et al., 2023)

## **2. Materials and Methods**

### **2.1 Preparation of *M. pudica* – Selenium nanoparticles**

Weigh 1g of *M. pudica* and mix it with 100 ml of distilled water. The mixture was boiled and then filtered to obtain the *M. pudica* solution. 0.356g of sodium selenite was measured and added to 50 ml of distilled water. Equal amounts of *M. pudica* solution and sodium selenite were mixed. After 24-48 hrs the *M. pudica* – Se NPs were synthesized.



Figure 1: Mimosa Pudica- mediated Se NPs

## 2.2 Cytotoxicity Assay :

### Brine shrimp Lethality Assay:

2g of iodine-free salt was weighed and dissolved in 200ml of distilled water. 6 well plates were taken and 10-12 ml of saline water was filled. To that 10 nauplii were slowly added to each well (5µL,10 µL,20 µL,40 µL,80 µL,100 µL). Then the Se- NPs nanoparticles were added according to the concentration level. The plates were incubated for 24 hours. After 24 hours, the plates were observed and noted for the number of live nauplii present and calculated by using the following formula,

number of dead nauplii/number of dead nauplii + number of live nauplii×100

## 2.3Antidiabetic Activity

### In-vitro antidiabetic assay

The in-vitro anti-diabetic assay was performed using two different techniques: The alpha-amylase inhibitory assay and the Alpha-Glucosidase Enzyme Inhibition assay.

#### 2.3.1 Alpha-amylase inhibitory assay

The inhibition of alpha-amylase was assessed by measuring the quantity of maltose released throughout the experiment. Various concentrations of *M. pudica*- Se NPs (10, 20, 30, 40, 50 µL) were mixed with 100 µL of α-amylase solution (1 U/mL) and incubated at room temperature for 30 minutes. An additional 100 µL of a starch solution with a concentration of 1% weight/volume was added to the combination. The mixture was then kept at room temperature for 10 minutes. 100 µL of a 96 mM solution of 3,5-dinitro salicylic acid (DNSA reagent) was added to the mixture to stop the reaction. The solution was then heated in a water bath for 5 minutes. Control was upheld by substituting an equivalent amount of enzyme extract with sodium phosphate buffer, which was kept at a pH of 6.9. The measurement of reading was recorded at a wavelength of 540 nm. Acarbose was used as a positive control.

The % inhibition was calculated using the formulae-

Where, C= control, T= test sample

### 2.3.2 $\beta$ -Glucosidase Inhibitory Assay

In the  $\beta$ -glucosidase enzyme inhibitory assay, Se NPs at concentrations of 10 to 50  $\mu\text{g/mL}$  were mixed and a starch solution (2% w/v maltose or sucrose) was added in the presence of 0.2 M Tris buffer at pH 8.0. The mixture was incubated at 37 degrees Celsius for 5 minutes. Subsequently, 1 mL of  $\beta$ -glucosidase enzyme (1 U/mL) was added, and the reaction was allowed to proceed at room temperature for 40 minutes. The reaction was terminated with the addition of 2 mL of 6 N HCl. Acarbose served as the positive control. The absorbance was measured at 540 nm using an ELISA plate reader and the percentage of  $\beta$ -glucosidase inhibition was determined by the formula:

% of inhibition =  $\frac{C-T}{C} \times 100$ , where C is the control and T is test sample.

## 3. Results

### 3.1 Cytotoxicity Assay

On Day 1, the survival rate of nauplii remained high across all concentrations. At the lowest concentration of 5  $\mu\text{g/mL}$ , 100% of the nauplii survived. Similarly, at concentrations of 10, 20, and 40  $\mu\text{g/mL}$ , the survival rates were also 100%. Even at the highest concentration of 80  $\mu\text{g/mL}$ , 100% of the nauplii survived. The control group, which did not receive any Se NPs, also exhibited a 100% survival rate.

On Day 2, a slight reduction in survival rates was observed at higher concentrations. At 5  $\mu\text{g/mL}$ , the survival rate remained at 100%. However, at 10, 20, and 40  $\mu\text{g/mL}$ , the survival rates decreased slightly to approximately 90%. At 80  $\mu\text{g/mL}$ , the survival rate further decreased to around 80%. The control group continued to exhibit a 100% survival rate.

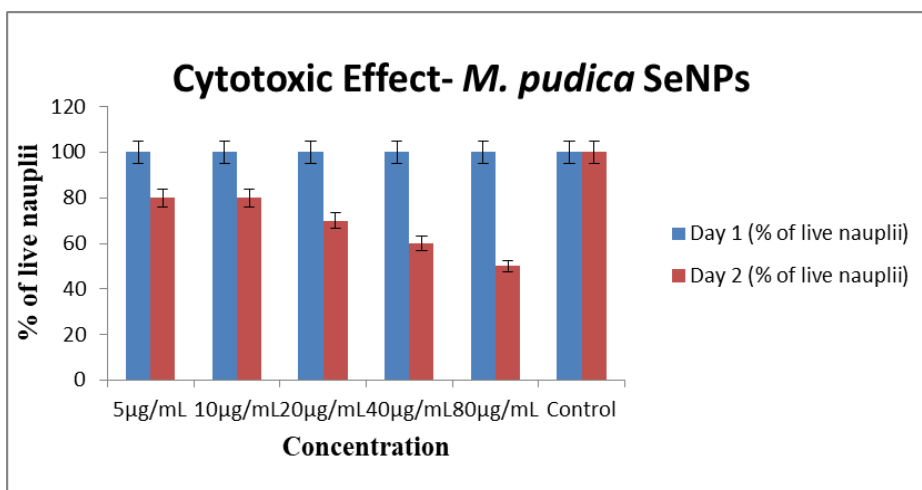


Figure 2: Cytotoxic effect of *M.pudica* – Se NPs

### 3.2 $\alpha$ -Amylase Inhibitory Assay:

The results of the  $\alpha$ -amylase inhibitory assay are presented in Figure 3 A). The *M. pudica*-mediated Se NPs exhibited a concentration-dependent increase in  $\alpha$ -amylase inhibition. At 10  $\mu\text{g/mL}$ , the inhibition was approximately 55%, which increased to about 65% at 20  $\mu\text{g/mL}$ , and further to 70% at 30  $\mu\text{g/mL}$ . At higher concentrations of 40 and 50  $\mu\text{g/mL}$ , the inhibition rates were around 75% and 80%, respectively. The standard drug showed slightly higher inhibition at corresponding concentrations, ranging from approximately 60% at 10  $\mu\text{g/mL}$  to around 85% at 50  $\mu\text{g/mL}$ .

### 3.3 $\beta$ -Glucosidase Inhibitory Assay:

The  $\beta$ -glucosidase inhibitory assay results, shown in Figure 3 B), also indicated a concentration-dependent inhibition by the *M. pudica*-mediated Se NPs. The inhibition at 10  $\mu\text{g/mL}$  was approximately 50%, which increased to about 58% at 20  $\mu\text{g/mL}$ , and to 64% at 30  $\mu\text{g/mL}$ . At 40 and 50  $\mu\text{g/mL}$ , the inhibition percentages were approximately 68% and 78%, respectively. The standard drug demonstrated a similar pattern, with inhibition rates ranging from around 55% at 10  $\mu\text{g/mL}$  to about 81% at 50  $\mu\text{g/mL}$ .

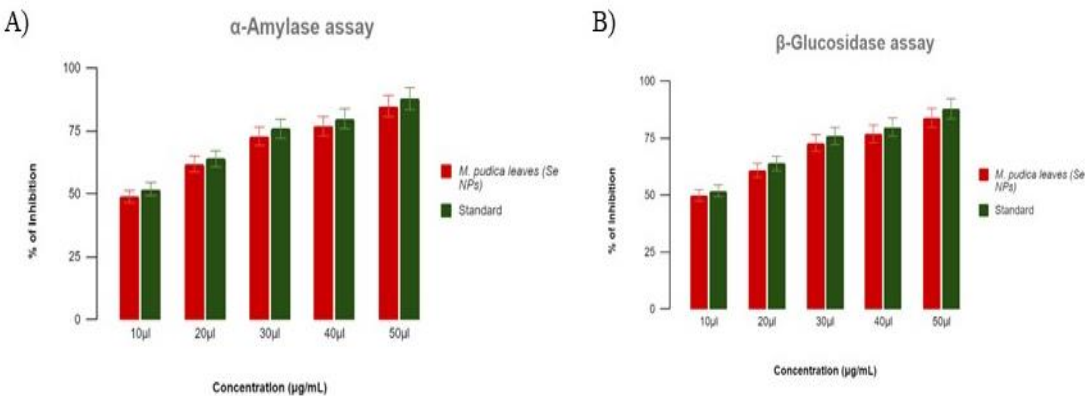


Figure 3: The antidiabetic activity of *M. pudica* – A) Alpha-amylase inhibitory assay B)  $\beta$ -Glucosidase Inhibitory Assay

## 4. Discussion

Brine shrimp lethality assay is a simple and easy procedure to test the cytotoxicity (Wu, 2014). The provided concentration range indicates the effects of a substance on cellular activity, specifically regarding cytoprotective and cytotoxic activities. Here's an expansion on the observations. (Figure 2)

Cytoprotective activity at 5  $\mu\text{g/mL}$  to 20  $\mu\text{g/mL}$  concentration: At concentrations ranging from 5  $\mu\text{g/mL}$  to 20  $\mu\text{g/mL}$ , the substance exhibits cytoprotective activity. This means that it has a beneficial effect on cells by protecting them from damage or promoting their survival under certain conditions. Cytoprotective activity can manifest in various ways, such as preserving cellular integrity, enhancing cell viability, or preventing cell death in the face of stressors or

detrimental factors.

Cytotoxic activity at 40 µg/ml and 80 µg/ml concentration: At higher concentrations of 40 µg/ml and 80 µg/ml, the substance demonstrates cytotoxic activity. Cytotoxicity refers to the ability of a substance to induce damage or cell death in living cells. In this context, the substance shows detrimental effects on cells at these concentrations. Cytotoxic activity can manifest in various forms, including cell death, inhibition of cell proliferation, disruption of cellular processes, or damage to cell structures.

As the concentration of a drug increases, the percentage of inhibition of the alpha-amylase enzyme increases as in the fig.3A). Alpha-amylase enzyme is an enzyme which will break down starch to glucose which the tissue can be absorbed easily (Yin et al., 2014). Hence the alpha-amylase is inhibited as almost equal to the standard drug acarbose, M.pudica- Se NPs may be an effective drug as an antidiabetic drug.

The percentage of inhibition of the Beta glucosidase enzyme increases the concentration of a drug increases as in fig3 B). Inhibition of Beta glucosidase will inhibit the breakdown of polysaccharides to monosaccharides which results in a reduction of blood glucose levels. (Van De Laar et al., 2005)

Hence the Beta glucosidase is inhibited as almost equal to the standard drug acarbose, the selenium nanoparticles mediated M. pudica- Se NPs may be an effective drug as an antidiabetic drug.

## 5. Conclusion

In conclusion, the study highlights the potential of Se NPs synthesized using M. pudica leaf extract as effective anti-diabetic properties. The green synthesis approach offers advantages such as sustainability, cost-effectiveness, and reduced toxicity. Further studies are necessary to these promising results into clinical applications, which may ultimately contribute to the development of novel therapies for managing diabetes mellitus.

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### Disclosure Statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## References

1. Bashary, R., Vyas, M., Nayak, S. K., Sutte, A., Verma, S., Narang, R., & Khatik, G. L. (2020). An Insight of Alpha-amylase Inhibitors as a Valuable Tool in the Management of Type 2 Diabetes Mellitus. *Current Diabetes Reviews*, 16(2), 117–136. <https://doi.org/10.2174/1573399815666190618093315>
2. El-Badri, A. M., Hashem, A. M., Batool, M., Sherif, A., Nishawy, E., Ayaad, M., Hassan, H. M., Elrewainy, I. M., Wang, J., Kuai, J., Wang, B., Zheng, S., & Zhou, G. (2022). Comparative

- efficacy of bio-selenium nanoparticles and sodium selenite on morpho-physiochemical attributes under normal and salt stress conditions, besides selenium detoxification pathways in *Brassica napus* L. *Journal of Nanobiotechnology*, 20(1). <https://doi.org/10.1186/s12951-022-01370-4>
3. Huang, Y., Yang, Y., Liang, B., Lu, S., Yuan, X., Jia, Z., Liu, J., & Liu, Y. (2023). Green Nanopesticide: pH-Responsive Eco-Friendly Pillar[5]arene-Modified Selenium Nanoparticles for Smart Delivery of Carbendazim to Suppress Sclerotinia Diseases. *ACS Applied Materials & Interfaces*, 15(13), 16448–16459.
  4. Song, J., Yu, S., Yang, R., Xiao, J., & Liu, J. (2023). Opportunities for the use of selenium nanoparticles in agriculture. *NanoImpact*, 31, 100478. <https://doi.org/10.1016/j.impact.2023.100478>
  5. L'Heveder, R., & Nolan, T. (2013). International Diabetes Federation. *Diabetes Research and Clinical Practice*, 101(3), 349–351. <https://doi.org/10.1016/j.diabres.2013.08.003>
  6. Khurana, A., Allawadhi, P., Singh, V., Khurana, I., Yadav, P., Sathua, K. B., Allwadhi, S., Banothu, A. K., Navik, U., & Bharani, K. K. (2023). Antimicrobial and anti-viral effects of selenium nanoparticles and selenoprotein based strategies: COVID-19 and beyond. *Journal of Drug Delivery Science and Technology*, 86, 104663. <https://doi.org/10.1016/j.jddst.2023.104663>
  7. Lovic, D., Piperidou, A., Zografou, I., Grassos, H., Pittaras, A., & Manolis, A. (2020). The Growing Epidemic of Diabetes Mellitus. *Current Vascular Pharmacology*, 18(2), 104–109. <https://doi.org/10.2174/1570161117666190405165911>
  8. Malayan, J., Selvaraj, B., Warriar, A., Shanmugam, S., Mathayan, M., & Menon, T. (2013). Anti-mumps virus activity by extracts of *Mimosa pudica*, a unique Indian medicinal plant. *Indian Journal of Virology*, 24(2), 166–173. <https://doi.org/10.1007/s13337-013-0143-2>
  9. Muhammad, G., Hussain, M. A., Jantan, I., & Bukhari, S. N. A. (2015). *Mimosa pudica* L., a High-Value Medicinal Plant as a Source of Bioactives for Pharmaceuticals. *Comprehensive Reviews in Food Science and Food Safety*, 15(2), 303–315. <https://doi.org/10.1111/1541-4337.12184>
  10. Elechi, N. N., & Unamba, N. O. E. (2023). Anti-diabetic activity of *Mimosa pigra* Linn (Fabaceae) methanol leaf extract on alloxan-induced diabetic rats. *GSC Advanced Research and Reviews*, 14(3), 016–020. <https://doi.org/10.30574/gscarr.2023.14.3.0069>
  11. Othman, M. S., Obeidat, S. T., Al-Bagawi, A. H., Fareid, M. A., Fehaid, A., & Moneim, A. E. A. (2022). Green-synthesized selenium nanoparticles using berberine as a promising anticancer agent. *Journal of Integrative Medicine*, 20(1), 65–72. <https://doi.org/10.1016/j.joim.2021.11.002>
  12. Elechi, N. N., & Unamba, N. O. E. (2023). Anti-diabetic activity of *Mimosa pigra* Linn (Fabaceae) methanol leaf extract on alloxan-induced diabetic rats. *GSC Advanced Research and Reviews*, 14(3), 016–020. <https://doi.org/10.30574/gscarr.2023.14.3.0069>
  13. Piacenza, E., Sule, K., Presentato, A., Wells, F., Turner, R. J., & Prenner, E. J. (2023). Impact of Biogenic and Chemogenic Selenium Nanoparticles on Model Eukaryotic Lipid Membranes. *Langmuir*, 39(30), 10406–10419. <https://doi.org/10.1021/acs.langmuir.3c00718>
  14. Wu, C. (2014, March 1). An important player in brine shrimp lethality bioassay: The solvent. PubMed Central (PMC). <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3960796/>
  15. Yin, Z., Zhang, W., Feng, F., Zhang, Y., & Kang, W. (2014).  $\alpha$ -Glucosidase inhibitors isolated from medicinal plants. *Food Science and Human Wellness*, 3(3–4), 136–174. <https://doi.org/10.1016/j.fshw.2014.11.003>
  16. Van De Laar, F. A., Lucassen, P. L., Akkermans, R. P., Van De Lisdonk, E. H., Rutten, G. E., & Van Weel, C. (2005). Alpha-glucosidase inhibitors for type 2 diabetes mellitus. *Cochrane Library*, 2009(1). <https://doi.org/10.1002/14651858.cd003639.pub2>
  17. Jayapriya Johnson, Rajeshkumar Shanmugam and Thangavelu Lakshmi A review on plant-



- mediated selenium nanoparticles and its applications J Popul Ther Clin Pharmacol Vol 28(2):e29–e40; DOI <https://doi.org/10.47750/jptcp.2022.870>
18. Kathiravan, A., Udayan, E., Rajeshkumar, S. et al. Unveiling the Biological Potential of Mycosynthesized Selenium Nanoparticles from Endophytic Fungus *Curvularia* sp. LCJ413. *BioNanoSci.* (2023). <https://doi.org/10.1007/s12668-023-01223-w>.
  19. Shanmugam R, Anandan J, Balasubramanian A K, et al. (December 20, 2023) Green Synthesis of Selenium, Zinc Oxide, and Strontium Nanoparticles and Their Antioxidant Activity - A Comparative In Vitro Study. *Cureus* 15(12): e50861. doi:10.7759/cureus.50861.
  20. Johnson J, Shanmugam R, Manigandan P (June 30, 2024) Characterization and Biomedical Applications of Green-Synthesized Selenium Nanoparticles Using *Tridax procumbens* Stem Extract. *Cureus* 16(6): e63535. doi:10.7759/cureus.63535.