

Impact of AgNPs on LLC cells

L. Zelena¹, G. Baranova¹, N. Tkachuk², S. Zahorodnia¹

¹ Department of Virus Reproduction, D.K.Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine, 03143 Kyiv, Ukraine

² Department of Biology and Human Health, T.H. Shevchenko National University “Chernihiv Colehium”, 14013 Chernihiv, Ukraine

INTRODUCTION & AIM

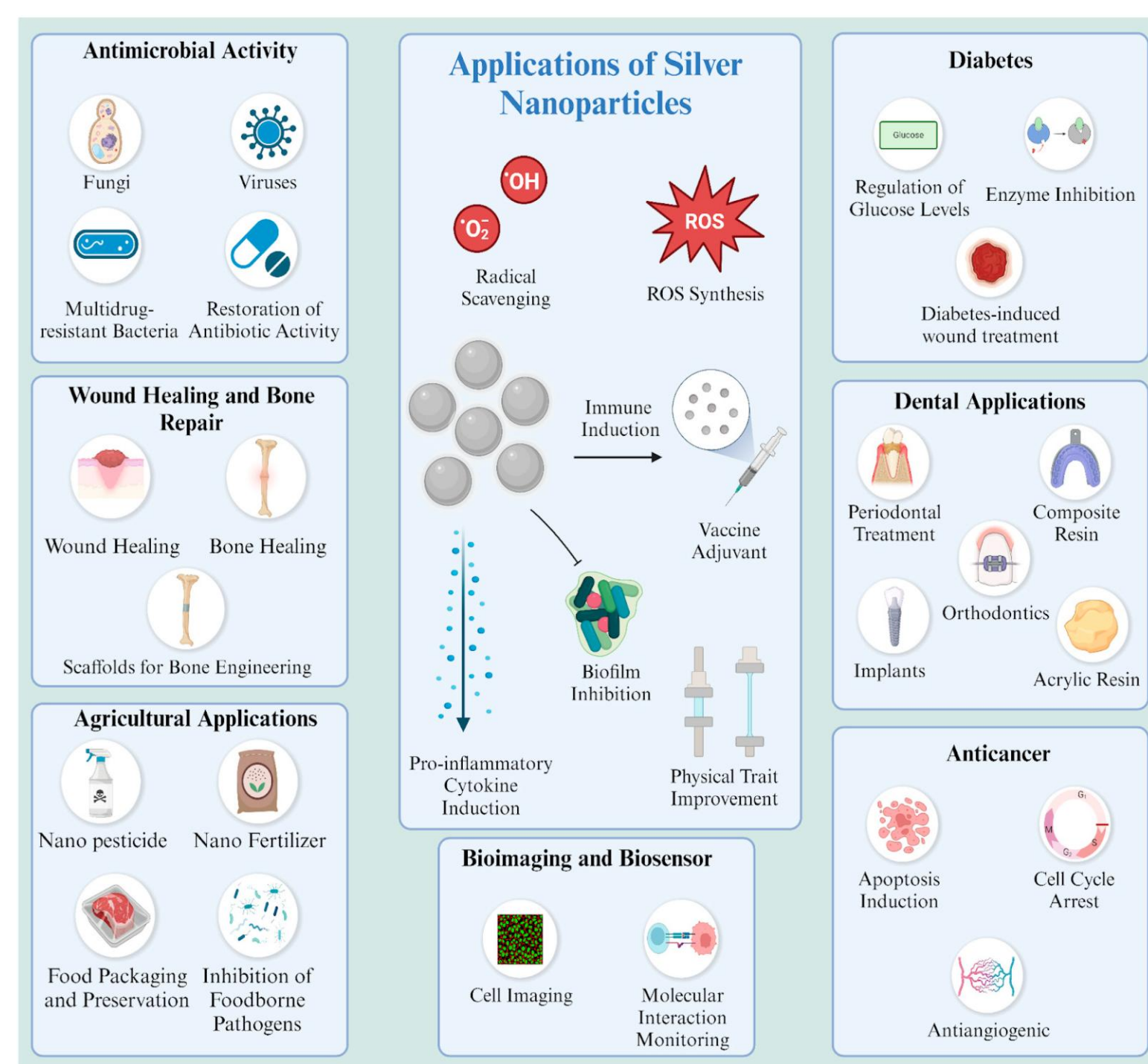


Figure 1. Applications of silver NPs [1]

Silver nanoparticles (AgNPs) are widely applied in various fields: medicine, pharmaceuticals, agriculture and others (Figure 1). They are suggested to exhibit antibacterial, antifungal, antiviral and anticancer activities. However, their effects depend on particle size, shape, physicochemical properties, synthesis method and target biological system. Lewis Lung Carcinoma (LLC) cells are an aggressive tumor model characterized by high oncogenicity and relative resistance to a range of anticancer drugs.

The purpose of this study was to assess the influence of AgNPs on LLC cells in terms of cytotoxicity, apoptotic gene expression and potential genotoxicity.

METHOD

• LLC cells were obtained from the Cell Bank of the R.E.Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of the NAS of Ukraine and were cultured according to standard methods.

• Colloidal silver concentrate coated with natural resin was manufactured by Noble Elements LLC (USA). Nominal particle size was 10 ± 2 nm and a silver concentration of 20000 ppm was used.

• Cytotoxicity of AgNPs was determined using the MTT assay. The studied concentration of NPs was 10–1000 ppm.

• Evaluation of apoptosis-associated gene expression was carried out with RT-qPCR analysis and

• PCR ISSR was performed to determine the genotoxicity effect.

RESULTS & DISCUSSION

Initial cytotoxicity screening established an IC_{50} value of 750 ppm for AgNPs against LLC cells. To investigate the molecular mechanisms driving this cytotoxicity, concentrations of 320 ppm and 640 ppm were selected for subsequent genotoxic and transcriptional analyses.

Analysis of apoptosis-related gene transcriptional activity showed downregulation of *bax*, *p53*, caspase 3 and 8 in treated with AgNPs samples (Figure 2). AgNPs at 640 ppm effectively abolished the expression of tumor suppressor *p53* and executioner Caspase 3 compared to controls. This was accompanied by a trace detection of Caspase 8 and a slight rebound in pro-apoptotic *bax*. Conversely, AgNPs at 320 ppm initiated standard *p53* downregulation and suppressed both *bax* and Caspase 8 transcription. *bcl-2* remained stable at all doses. Overall differences in gene expression did not reveal an apoptosis-related pathway for nanoparticle-induced cell death

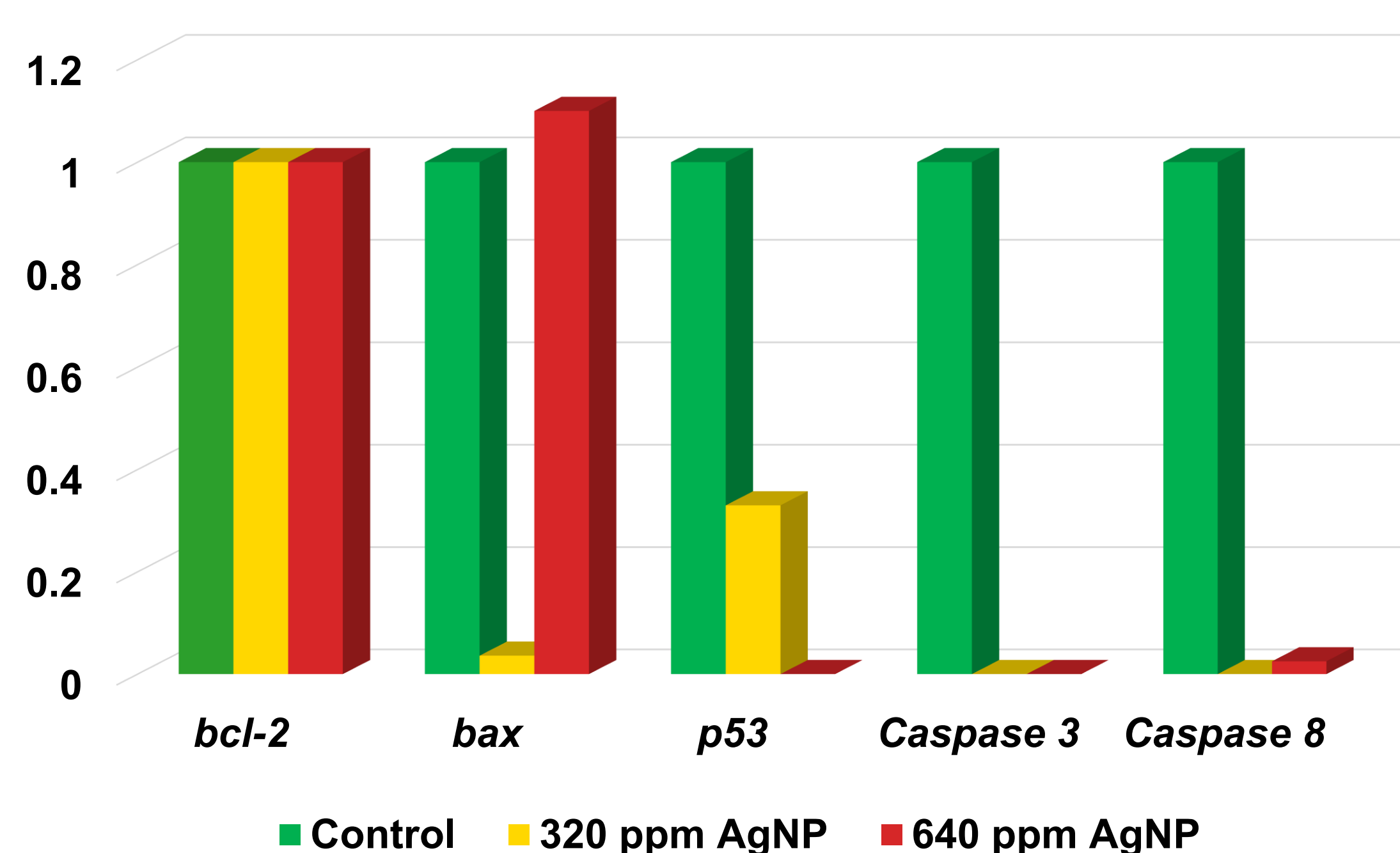


Figure 2. Diagram of relative gene expression ($2^{-\Delta\Delta C_t}$)

To assess the possible influence of AgNPs on DNA, PCR with primers targeting di- and trinucleotide repeats was carried out (Figure 3). Comparative analysis of the amplicon patterns revealed that the size range of PCR fragments varied from 300 bp to 3000 bp, and differences between control and treated samples were observed. The most pronounced changes, characterized by the absence of certain amplicons in the banding profile, were detected in LLC cells treated at 640 ppm.

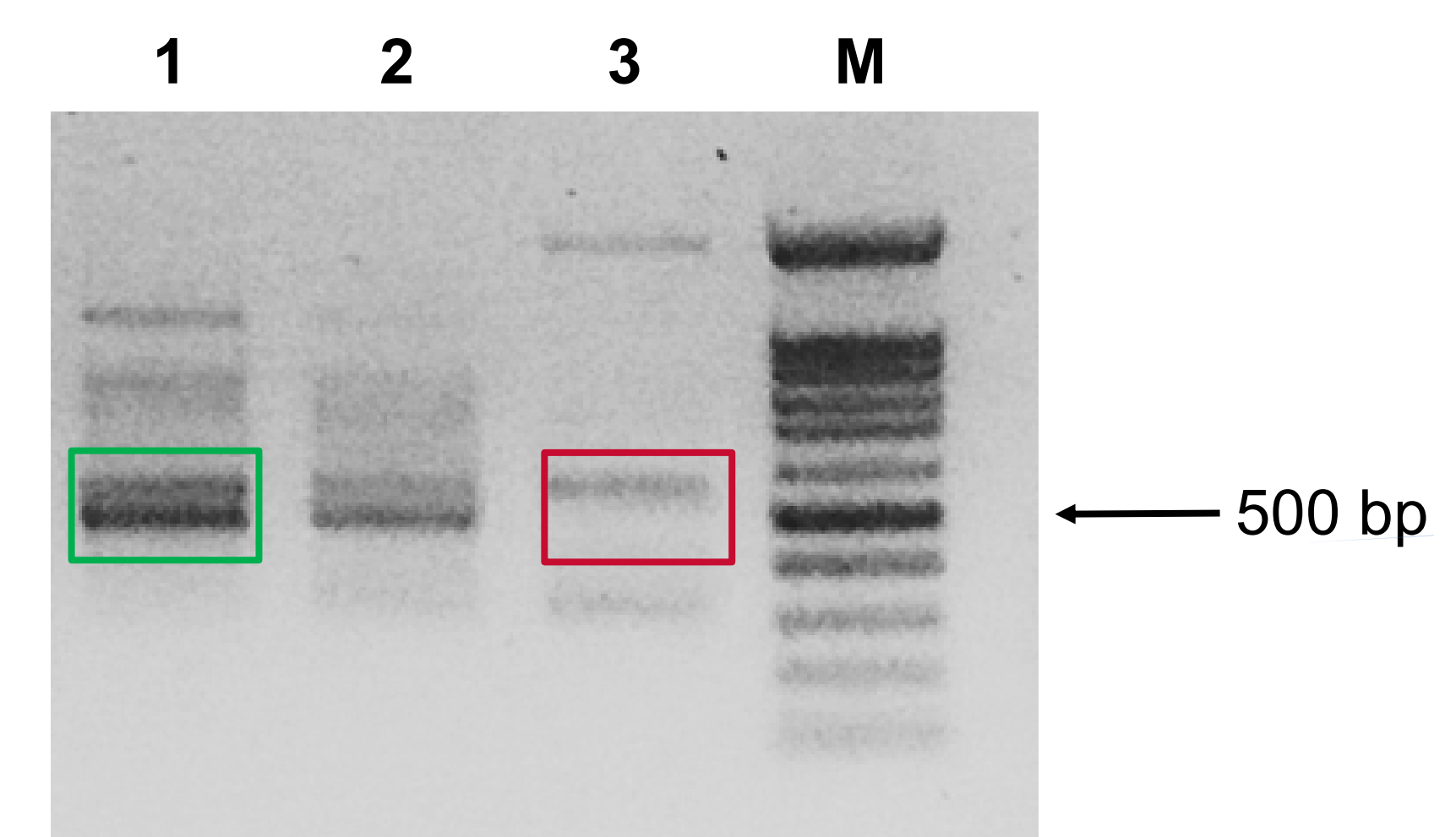


Figure 3. Electrophoregram of PCR products. M - DNA ladder, 1 - control, 2 - treatment with 320 ppm, 3 - treatment with 640 ppm

CONCLUSIONS

Potent Cytotoxicity: Colloidal silver nanoparticles (AgNPs) demonstrate significant, dose-dependent cytotoxicity against Lewis Lung Carcinoma (LLC) cells, with an established IC_{50} of 750 ppm.

Apoptotic Failure: Transcriptional profiling confirms that AgNP-induced cell death bypasses classical apoptotic pathways, establishing suppression of *p53*, Caspase 3, and Caspase 8 expression at higher concentrations.

Alternative Mechanisms: The results obtained by PCR ISSR suggest that the inhibitory effect on LLC viability is driven by alternative pathways, most likely involving severe oxidative stress responses and potential necrosis.

REFERENCES

- Eker, F.; Duman, H.; Akdaşçi, E.; Witkowska, A.M.; Bechelany, M.; Karav, S. Silver Nanoparticles in Therapeutics and Beyond: A Review of Mechanism Insights and Applications. *Nanomaterials* **2024**, *14*, 1618. <https://doi.org/10.3390/nano14201618>
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