ORIGINAL ARTICLE

Cytotoxicity Evaluation of Silica Nanoparticles and Antibiotic-coated Variants using a Brine Shrimp Lethality Assay

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Abstract

Aim: Nanotechnology has become a promising field in different branches of science, including medicine. Silica nanoparticle (NP) has unique characteristics, and due to this reason, the potential applications of silica NPs in drug delivery and therapeutics have been widely studied. However, concerns have been raised about their cytotoxic effects. This study was performed to investigate the cytotoxicity effects of silica NPs and silica NPs coated with antibiotics by the Brine Shrimp Lethality assay. **Materials and Methods:** In this assay, the saline solution was prepared in a six-well end-loaded split plate with a single 10 brine shrimp nauplii in each well. Various levels of NPs and their antibiotic-coated counterparts were added to the wells. The plates were incubated at room temperature for 24 h after which the number of viable nauplii was determined. The collected data were analyzed. One-way analysis of variance and *post hoc* tests were used to test the statistical significance. **Results:** Cytotoxic effects of different concentrations of nano-silica mixed with different antibiotics were evaluated. In the 5 uL concentration, there was a non-significant difference in all the groups ($P > 0.05$). At the 10 µL concentration, nanosilica ciprofloxacin had the least survival rate which is statistically significant compared to other groups ($P < 0.000$). At the highest concentration of 15µL, nanosilica ciprofloxacin showed the lowest survival rate which is statistically significant compared to other groups ($P < 0.000$). **Conclusion:** The results of this study indicate that even at high concentrations, silica NPs and its antibiotic-coated variants show very low cytotoxic effects on brine shrimp nauplii. These findings offer useful information for the potential biomedical uses of silica NPs and their antibiotic-coated variants.

Key words: Antibiotic-coated variants, brine shrimp lethality assay, cytotoxic effects, innovative, nanotechnology, silica nanoparticles, survival rate

INTRODUCTION

The silica nanoparticles (NPs) have outstanding qualities making them useful in an extensive variety of biomedical work. These NPs are non-toxic and easily customizable as they can be produced in different pore structures.[1] They provide accurate control of their physicochemical surface characteristics, and they are multifunctional materials in the field of biomedical use.[2] The antimicrobial agents can be carried by silica NPs, which allows a specific drug delivery, decreases the frequency of dosing, and improves the efficiency of pharmacokinetics.[3] Their minimal toxicity and ability to act as versatile molecules, e.g., light harvesting and signal amplification, are indispensable to such applications as medical diagnostics and therapy.[4] Moreover, silica-coated magnetic NP has a

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Received: 22-02-2026
Revised: 20-03-2026
Accepted: 30-03-2026

better dispersibility and acid resistance without losing their magnetic activity, and hence, they can be applied in various tasks such as catalysts and drug delivery, magnetic resonance imaging.[5] Silica NPs have a lot of uses in the dentistry profession such as drug delivery, drug target systems, magnetohyperthermia, and magnetic resonance imaging.[6] Magnetic NPs made of silica possess a high biocompatibility rate, stability, and ease of functionalization, which makes them more suitable for different dental applications.[7] NPs, in the context of regenerative dentistry, have been tweaked to be more effective in antimicrobial activity and tissue regeneration with the end result to be better prognosis after a period of time.[8] Another contribution of silica NPs to oral health is in the diagnosis, prevention, and treatment of oral diseases; improvement of the properties of dental prostheses and implants; and delivery of drugs through the oral mucosa in the body.[9] The silica-based nanomaterials have good dental properties such as improved physical and mechanical properties and therapeutic bone properties. In the field of dentistry, NPs, also called nano dentistry, have potential promises of remineralization, antimicrobial, local anesthesia, antimicrobial, antimicrobial, anti-inflammatory, osteoconductivity, and stem cell differentiation.[10] As much as silica NPs have enormous potential in dentistry, owing to their peculiar characteristics such as controllable size, large surface area, and biocompatibility, there are also a number of obstacles associated with it. Such difficulties involve the fact that they require further studies to know more about their properties since some of them remain unexplained to a better degree.[11] Moreover, silica NPs may prove to be quite harmful to dental cells, and thus, the toxicity of these NPs needs to be properly investigated.[12] It can also be improved in the field of addressing the capability of silica NPs to resist intraoral forces. The challenges highlighted the need to conduct continuous research and come up with measures that would help to maximize the use of silica NPs in dentistry. Research studies on silica NPs in recent years have involved their use in crop insect pests, as adsorbents of wastewater pollutants, as nanocarriers of biomedicine, and as therapeutic nanodevices in cancer treatment. Silica NPs have also been shown to induce tolerance to insect pests in crops and to translocate easily into plant tissues to improve plant defense systems.[13] They have special structure attributes that render them good adsorbents of contaminants in wastewater.[14] Silica NPs are seen as a possible solution to both drug delivery systems through nanocarriers that can react to a particular stimulus and also as imaging nanoplatforms in the world of biomedicine.[15] There has also been development of silica-based functional composite to be used as therapeutic nanodevices in the treatment of cancer.[16]

MATERIALS AND METHODS

Cytotoxic effect – brine shrimp lethality assay

Cytotoxicity of silica NPs and antibiotic-coated silica NPs was assessed by the use of a Brine Shrimp lethality assay. The saline solution was ready by weighing 2 g of the iodine-free salt accurately and dissolving it in 200 mL of distilled water. Then, 10–12 mL of this saline solution was poured into 6-well enzyme-linked immunosorbent assay (ELISA) plates. The introduction of 10 nauplii was done in every well. Various concentrations of NPs and their antibiotic-coated counterparts were added as per the specified concentration levels. The loaded plates were then left to incubate at room temperature for a duration of 24 h. Following this incubation period, the ELISA plates were scrutinized, and the count of viable nauplii was determined using the following formula.

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

Statistical analysis

The collected data were analyzed and presented. One-way analysis of variance and *post hoc* tests were used to assess the statistical significance when comparing within-group and between-group differences using IBM Statistical Package for the Social Sciences Statistics for Windows, Version 23.0 (IBM Corp., Armonk, NY).

RESULTS

The cytotoxic effects of various concentrations of nano-silica in combination with different antibiotics (doxycycline, ciprofloxacin, metronidazole, and triple antibiotic) were evaluated by comparing the survival rates of brine shrimp nauplii on day 2. In the control, which did not contain nano-silica or antibiotics, all brine shrimp nauplii survived, with a survival rate of 100%. In the group treated with 5 μL of nanosilica combined with various antibiotics, all concentrations resulted in a survival rate of 100%. This suggests that even at this low concentration, there was no observable cytotoxic effect on the brine shrimp nauplii compared to the control group. No significant difference was observed in any of the groups ($P > 0.05$). At the 10 μL concentration, nano-silica doxycycline, nano-silica metronidazole, nano-silica triple antibiotic paste, and the control group exhibited a survival rate of 100%; however, nano-silica ciprofloxacin showed 84.67% survival rate which was slightly lesser than the other groups. Nanosilica ciprofloxacin showed the least survival rate which is statistically significant when compared to other groups ($P < 0.000$); however, the combination with triple antibiotics maintained a survival rate of 100%, indicating no significant cytotoxicity. At the highest concentration of 15 μL of nanosilica, the doxycycline and control group exhibited a 100% survival rate, nanosilica ciprofloxacin showed a 66.33% survival rate, nanosilica metronidazole showed 87.67% survival rate, and nanosilica triple antibiotic paste showed 84.33% survival rate. Nanosilica ciprofloxacin showed the lowest survival rate which is statistically significant when compared to other groups ($P < 0.000$) [Table 1 and Figure 1].

Table 1: The cytotoxic effects of various concentrations of nanosilica combined with different antibiotics were evaluated. At 5 μL concentration, no significant difference was observed in any of the groups ($P>0.05$). At the 10 μL concentration, nanosilica ciprofloxacin showed the lowest survival rate which is statistically significant compared to other groups ($P<0.000$). At the highest concentration of 15 μL, nanosilica ciprofloxacin showed the lowest survival rate which is statistically significant when compared to other groups ($P<0.000$)

Groups	5 μL	10 μL	15 μL
Nano silica doxycycline			
Mean	100	100	100
Standard deviation	0.00	0.00	0.00
Nano silica ciprofloxacin			
Mean	100	84.67	66.33
Standard deviation	0.00	1.53	1.53
Nano silica metronidazole			
Mean	100	100	87.67
Standard deviation	0.00	0.00	1.53
Nano silica triple antibiotic paste			
Mean	100	100	84.33
Standard deviation	0.00	0.00	3.05
Control group			
Mean	100	100	100
Standard deviation	0.00	0.00	0.00

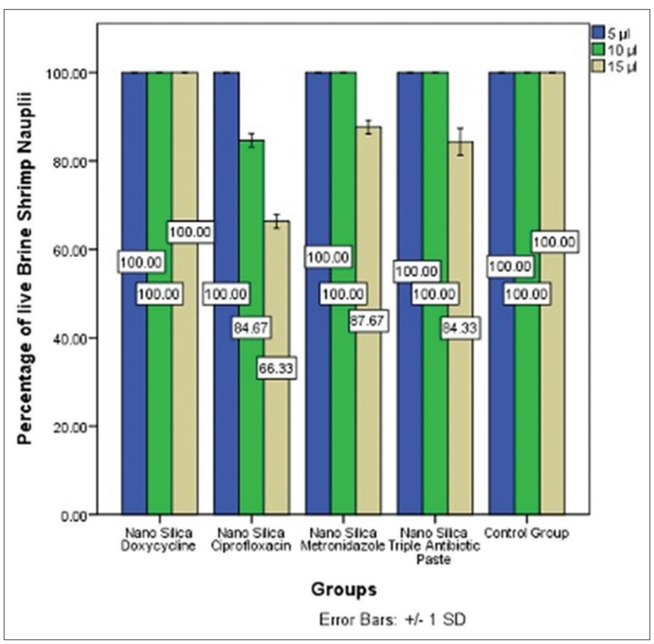

Figure 1: The graph illustrates the proportion of live brine shrimp nauplii of all the groups under different concentrations

DISCUSSION

The creation of new technologies has enabled the production of silver NPs with exact target functions, dimensions, and forms. These NPs can be functionalized with molecular capping agents with the aid of mediating agents such as bacteria and plants. This allows for a variety of uses for the NPs in the field of dentistry, particularly with regard to their antimicrobial efficiency.[17] The Brine Shrimp Lethality assay is a widely used method for assessing the cytotoxic effects of various substances on living organisms. In this case, the study aimed to evaluate the cytotoxicity of nano-silica and its antibiotic-coated variants on brine shrimp nauplii. In the experiment, various concentrations of nano silica and antibiotics were exposed to the nauplii, and survival rates were measured after incubating the animals for 24 h. These findings showed different degrees of cytotoxicity that can be useful in determining the possible dangers of these NPs and combinations of antibiotics.

The total of brine shrimp naupli survived in the control group with no addition of nano-silica and antibiotics showed a 100% survival rate. This outcome is used as a critical guideline to determine the level of cytotoxicity of the tested substances. The cytotoxic side of the treatments can be attributed to any variation of this 100% rate of survival. At a low concentration of 5 μL, it was observed that nano-silica combined with various antibiotics did not significantly affect the survival of brine shrimp nauplii, with all concentrations resulting in a 100% survival rate. This suggests that at this minimal exposure level, there was no observable cytotoxic effect on the brine shrimp nauplii compared to the control group. It is important to highlight that these results are promising, indicating that these low doses are relatively safe. At the moderate concentration of 10 μL, nano-silica combined with doxycycline, ciprofloxacin, and metronidazole exhibited a survival rate of 80%, which was slightly lower than that of the control group. However, the combination with triple antibiotics maintained a survival rate of 100%, indicating no significant cytotoxicity. These results suggest that, at this concentration, there may be a mild cytotoxic effect with doxycycline, ciprofloxacin, and metronidazole when combined with nanosilica, but it remains within acceptable limits. On the other hand, the combination with triple antibiotics seemed to be well-tolerated by the brine shrimp nauplii, as it did not significantly differ from the control group's survival rate.

The highest concentration of 15 μL of nano-silica combined with doxycycline, ciprofloxacin, and metronidazole resulted in decreased survival rates of 60% for doxycycline, 80% for ciprofloxacin, and 80% for metronidazole. The combination of triple antibiotics also showed a survival rate of 80%. These concentrations exhibited reduced survival compared to the control group. The reduced survival rates at this increased concentration would show that there is a dose-dependent cytotoxic effect. Interestingly, doxycycline was the most cytotoxic at this concentration, and ciprofloxacin and metronidazole were slightly less but significantly cytotoxic. The triple antibiotic combination still showed a higher tolerance to the single antibiotics, indicating further that combinations may be effective in decreasing the cytotoxicity.

These findings of the Brine Shrimp Lethality assay suggest that nano-silica at low concentration levels is not very toxic in brine shrimp nauplii. Nevertheless, cytotoxicity is enhanced with the rise in concentration of nano-silica when combined with certain antibiotics. It is also paramount to take into account that the selection of an antibiotic is a contributing factor to cytotoxicity. The cytotoxic effect of doxycycline was the greatest at the highest concentration then ciprofloxacin and metronidazole. On the contrary, the triple antibiotic combination demonstrated a better safety profile. These results have implications for the possible applications of nano-silica and antibiotic-coated nano-silica in other applications. To achieve the desired therapeutic effect and at the same time, prevent the possible cytotoxicity, it is important to balance between the desired therapeutic effect and the possible cytotoxicity, particularly when using higher concentrations of nano-silica. Furthermore, more research is required to understand the mechanisms behind the cytotoxicity and to discuss possible measures to reduce the negative outcome.

Silica NPs have shown great potential in applications in endodontics. They are also desirable due to their special properties such as increased ability to combat endodontic diseases through heightened antibacterial activity, reactivity, and functionalization ability, which enable them to be added to obturation materials and sealers.[18] Silica NPs have also been shown to be useful because of their attribute of controlled release of bioactive compounds and improved biophysical properties of scaffolds employed in regenerative endodontics. It should be remembered, though, that even with these favorable features, the cytotoxicity of silica NPs as far as endodontic disease treatment is concerned is still an issue of concern.[19]

The effect of silica NPs in relation to cytotoxicity is conjectured to have been examined in several studies. An example is the study of Betancourt *et al*.[20] who found that silica NPs such as SiO_2 were dose and time-dependent cytotoxic on pulp stem cells of the dental tissue. Concerns of how silica NPs may negatively impact the cellular functions of human beings and animals were also raised by Hayat *et al*.,[7] who pointed out that silica NPs may affect biochemical and epigenetic processes. Due to their different modes of action through which they act to cause cell lysis such as attacking the peptidoglycan of the cell wall and cell membrane, inhibiting the production of bacterial proteins, and disrupting the bacterial DNA along with their ability to generate oxidative stresses through the production of free radicals, NPs have become more popular in literature.[21,22] To realize the potential of silica NPs in endodontics, more studies are much needed to counter these fears. Further studies are required to learn more about the cytotoxicity of silica NPs and find ways to obtain safe and effective methods of treating endodontic diseases. Such information will be critical in the process of ensuring that the advantages of silica NPs can be fully reaped in the endodontics sector with the least harm to the patient's health.[5]

One of the possible strategies in combating periodontal infections, in particular, *Enterococcus faecalis* that causes periodontitis, is the combination of NPs and antibiotics.[23] On the whole, the present research is a valuable source of information concerning the cytotoxicity of nano-silica and antibiotic-coated versions[24] on brine shrimp nauplii with the focus on dose-dependent effects and antibiotic selection. The research findings add to the current debate on the safe and optimal application of NPs and nanomaterials in the medical and environmental fields.

CONCLUSION

The study was intended to compare the cytotoxicity of silica NPs with that of antibiotic-coated particles on a Brine Shrimp lethality assay. Its findings showed that these NPs, even at high concentrations, had low cytotoxic effects on brine shrimp nauplii. The given finding is a major discovery because it is possible to implement these NPs in the biomedical field, especially in drug delivery systems because they are seemingly non-toxic. It was however noted that with increased levels, there was a mild reduction in the survival rates, especially when the NPs were mixed with some antibiotics. This means that though the NPs are not necessarily very toxic, their interaction with other substances may lead to their toxicity. These results indicate the need to conduct additional studies in this field. Further research in the relationship between these NPs and other antibiotics should be conducted to verify these findings and determine the interactions between the two. Besides, studies should also be conducted on the possible uses of these NPs in medicine, especially on the development of drug delivery systems. To sum up, although this paper offers good preliminary evidence about the cytotoxic properties of silica NPs and antibiotic-coated silica nitrate NPs, the future of these NPs in biomedical research and practice needs to be studied in more detail to clearly comprehend the prospective advantages and dangers.

AUTHOR CONTRIBUTION

Conceptualization: MSH, DPA, RS. Data curation: MSH. Formal analysis: MSH, DPA, RS. Funding acquisition: MSH. Investigation: MSH, DPA. Methodology: MSH, DPA. Project administration: MSH, DPA, RS. Resources: MSH, RS. Supervision: MSH, DPA, RS. Validation: MSH, DPA, RS. Visualization: MSH, DPA, RS. Writing-original draft: MSH, DPA, RS. Writing - review & editing: MSH, DPA, RS

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data are accessible from the corresponding author upon reasonable request

ETHICAL APPROVAL

The study has been approved by the scientific review board with the reference number SIMATS/Ph.D. Regn./A4/2019/0186

FUNDING/SUPPORT

This research received no specific grant from public, commercial, or not-for-profit funding agencies.

ACKNOWLEDGMENT

We would like to acknowledge the Gold lab at Saveetha Dental College.

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Source of Support: Nil. **Conflicts of Interest:** None declared.