Original Article

Effect of Adding Selenium Nano Particles on Improving Silver Diamine Fluoride- Antibacterial Efficiency, Cell Viability, and Derived Tooth Discoloration in Vitro

Maitha Sameer Kadhim[1]*, Shammaa Anees Sahib AlAnsari[2], Safa Salam[3], Areej Abdalghfour[4]

[1]Department of Pedodontics and Preventive Dentistry, Al-Rafidain University College, Baghdad, Iraq.
ORCID iD: https://orcid.org/0000-0002-2098-1753, Email: maitha.alnasri@ruc.edu.iq
[2]Department of Conservative Dentistry, College of Dentistry, Ibn Sina University of Medical and Pharmaceutical Science, Baghdad, Iraq.
ORCID iD: https://orcid.org/0000-0002-5152-9663, Email: shammaa.alansary@ibnsina.edu.iq
[3]Department of Conservative Dentistry, College of Dentistry, Ibn Sina University of Medical and Pharmaceutical Science, Baghdad, Iraq.
ORCID iD: https://orcid.org/0009-0000-7145-4817, Email: safa@ibnsina.edu.iq
[4]Department of Periodontics, Al-Esraa University College of Dentistry, Baghdad, Iraq.
ORCID iD: https://orcid.org/0009-0003-9072-4191, Email: areej@esraa.edu.iq

Abstract

Background: The discipline of dentistry consistently pursues novel materials that can optimise treatment results and augment patient comfort. Selenium nanoparticles exhibit potential due to their unique properties, including the ability to combat dangerous oral flora and their safety, as they are a consistently beneficial metal for the human body. All of these attributes affect cellular repair and regeneration. The integration of these nanoparticles into the advantageous Silver Diamine Fluoride (SDF) filling material may represent a viable strategy to reduce cariogenic activity and root canal infections as a novel modification of SDF material. **The Study Aims:** To improve SDF restoration material by integrating selenium nanoparticles into three distinct combinations; these nanoparticles are recognised for their capacity to mitigate inflammation and oxidation. The objectives are to examine the impact of Se Nanoparticles (NPs) on antibacterial efficacy and cytotoxicity, and subsequently assess the optical properties of SDF in isolation by comparing them with those of three additional additives, with the goal of determining whether the incorporation of these antioxidant metals could modify the dark imprint on dental lesions. **Material and Methods:** Four tested groups were prepared (mix one \ 3% Selenium Nanoparticles, mix two \ 4% Selenium Nanoparticles, mix three \ 5% Selenium Nanoparticles) and SDF alone as a control group. To measure the antibacterial activity against three different human pathogens — Enterococcus faecalis, Lactobacillus, and Streptococcus — we used the Muller-Hinton agar diffusion method, measuring the inhibition zone diameter in millimeters. The collected data were statistically examined using a t-test. $P < 0.05$ was picked as the significant threshold. In line with ISO-10993-5 rules, we also used the MTT test to see how active the fibroblast cells were after 24, 48, and 72 hours, related to cytotoxicity measurement. Finally, a spectrophotometer analysis was used to evaluate the absorption in wavelengths ranging from 190 to 780 nm. **Results:** All three formulations of SDF-Se nanoparticles (3%, 4%, and 5%) exhibited superior antibacterial efficacy compared to SDF alone, with optimal inhibition zones attained at a concentration of 5% Se nanoparticles. This indicates a promising antibacterial potential against gram-positive rods and cocci, making them suitable candidates for caries-arresting therapies in both coronal and radicular cavities. The cytotoxicity investigation indicates that SDF and its combinations demonstrate time-dependent impacts on cell viability. The spectrophotometric test simultaneously indicates that the gathered data were uncorrelated with the addition of Se nanoparticles. **Conclusion:** The integration of Selenium Nanoparticles markedly enhances the antibacterial efficacy of SDF dental restoration while maintaining acceptable cell viability. However, the addition of these nanoparticles does not ameliorate SDF discolouration through an antioxidant effect, contrary to the hypothesis that substituting or incorporating metals like selenium would diminish the dark hue of silver oxides as a chemical by-product.

Keywords: Caries, Silver Diamine Fluoride, Selenium Nanoparticles, Enterococcus Faecalis, *Lactobacillus, Streptococcus*, MTT Test.

Introduction

ECC stands for early childhood caries, which pertains to carious lesions in one or more teeth, with or without cavities, as well as extracted teeth lost due to caries, including primary repaired teeth in children under six years of age.

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Address for Correspondence: Escuela Superior Politècnica de Chimborazo, (ESPOCH) Sede Orellana, El Coca 220150, Ecuador
Email: santiagoa.guaman@espoch.edu.ec

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[1] Young children, because to their immaturity, limited capabilities, and diminished willingness to comply with dental professionals, frequently exhibited such behaviours when seated in the dental chair. Particularly following the COVID-19 epidemic, during which their parents deferred clinical appointments as a preventative measure.

In contemporary dentistry, the advancement of conservative and preventative practices necessitates the formulation of a bioactive substance endowed with therapeutic qualities. Recently, Silver Diamine Fluoride (SDF) has been presented as a non-invasive and economical dental care alternative, obviating the necessity for surgical intervention or therapy. Silver diamine fluoride (SDF) is commonly employed to address dental caries in paediatric patients.[2] It mostly consists of water, silver, ammonia, and fluoride. Silver ions impede pathogenic cellular proliferation by interacting with the cell wall, penetrating intracellular components, and disrupting bacterial metabolic and replication capabilities.[3] In the subsequent section, ammonia elevates the pH and stabilises the environment, whereas fluoride efficiently combats bacteria and reconstructs tooth structure using minerals. A six-month treatment of 38% SDF has demonstrated the ability to halt approximately 81% of active dentin caries.[4] In the absence of severe adverse effects, including toxins or systemic damage. Operators and parents remain opposed to the application of SDF on children's teeth. It may be due to its unesthetic appearance and the discolouration of the dentinal structure to black. In actuality, silver ions convert into metallic silver and silver oxides, resulting in a darker appearance of SDF. This results in the adherence of protein and phosphate silver complexes to the dental structure.[5]

SDF exhibits superior efficacy as a topical anti-carcinogenic agent, even exceeding the effectiveness of fluoride varnish treatment, as evidenced by multiple in vitro and in vivo studies. SDF surpasses fluoride patches and mouth lotions in preventing the exacerbation of sores and facilitating increased fluoride absorption into the dental structure.[6]

The position of teeth significantly influences parents' decisions regarding their children's acceptance of silver diamine fluoride (SDF) therapy, particularly due to its low acceptance rate for anterior teeth.[7] School-age children who have SDF treatment on anterior teeth primarily experience bullying and psychological trauma from their peers.[8]

Consequently, there is an immediate necessity to identify an alternate substance to substitute or enhance silver ions with antibacterial capabilities, thereby diminishing the propensity for carious lesions to darken.

Nanomaterial-derived antimicrobials are currently attracting considerable attention in the medical field. It pertains to possessing distinctive characteristics, including an extensive surface area and an accelerated response time.[5,9] Selenium nanoparticles are one of these. They are secure and have garnered significant attention in biological research due to their ability to eliminate several types of pathogens through their antioxidant, antibacterial, antifungal, and antiviral capabilities. Nevertheless, little research has been undertaken to evaluate its efficacy as a bactericide and its protective effects on human cell proliferation.[10,11]

Materials and Methods

Incorporation of Nano Selenium Particles into SDF (silver diamine fluoride) Material

- All chemicals and reagents used in this work were of high purity for analytical purposes. The dental materials used in this research complied with the requirements for human use. A silver diamine fluoride (SDF) liquid (India: kids-e-dental LLP) was used as a base material in this experiment.
- The Nano selenium with purity of 99.9% with a molecular weight of 78.96 g/mole applied in this work was supplied from Nanoshel LIC 3422, Old Capital Suite 1305, Wilmington, DE 19808, United States, with a particle size of <80 nm.

All preparations were performed under sterile conditions to avoid any sample contamination. The three mixtures (mixture one: 3%, mixture two: 4%, and mixture three: 5%) were prepared by weighing an exact amount of Selenium nanoparticles, followed by vigorous mixing with SDF to achieve the required percentage of Se Nano-SDF.

Microbiological Analysis

The agar diffusion test (ADT) on Muller-Hinton medium (BD France) is lied on to measure the diameter in millimeter of the bacterial inhibition zones towards three different gram-positive rods and cocci species; *Enterococcus faecalis*, (a gram-positive, facultative anaerobic coccus), *Lactobacillus* (Gram-positive, Obligate anaerobes rods) and *Streptococcus* (Gram-positive Facultative anaerobes cocci). For the preparation of the bacterial culture and determination of the inhibition zone, the bacterial species suspected in our study was isolated and grown in a nutrient broth (Biolife USA), then incubated at 37 °C for approximately 18 to 24 hours. A volume of 0.1 millimeter from the suspension of bacterial species was spread on the agar nutrient surface waiting for 24 hours at 37º C. a test tube filled with 5ml of normal saline mixed with addition of a single colony to yield modest turbid bacterial suspension that linked to standard turbidity solution almost closed to 1.5 $\times 10^8$ CFU/ml. A portion of the bacterial suspension was carefully spread on Mueller-Hinton agar medium using a sterile cotton swab and left for ten minutes. Wells of 5 millimeters were applied in the previous agar layer and organized as three wells per plate. The discs of agar in 50 µl of purified and cured EPS were added to each well through a micropipette, and the D.W. was then added to the well at the midpoint as a control. Incubation of these plates at 37 ºC for about 18 hours. This procedure was completed by recording the diameters of inhibition zones.

Cytotoxic Activity Testing

This now makes it evident that the reduction of tetrazolium salts is a reliable method for determining how quickly cells divide. A chemical that scientists use, MTT, to check the viability of cells. MTT is reduced metabolically by cells that are alive and proliferating, and its chemical formula is (3-(4,5-dimethylthiazolyl-2)-, 5-diphenyltetrazolium bromide). There are several ways in which dehydrogenase enzymes can facilitate this metabolic reduction. Spectrophotometry can be used to separate and measure the purple formazan produced by cells. The study kit consists of two parts: 12.5 ml of MTT reagent should be stored at 24°C, and 2.125 ml of washing reagent should be stored at room temperature. The basic protocol: The MTT Reagent is ready for use immediately. At 4°C and in the absence of light, it will remain stable for up to 18 months. Each time, the correct amount should be withdrawn and placed into a clean tube. After that, the stock bottle should be stored at 4°C in the dark. In each well, between 1,000 and 100,000 cells were put down. They were then left to grow for 6 to 24 hours. After 10 µL of MTT Reagent was added, it was left to sit for 2 to 4 hours, or until a purple precipitate could be observed. After that, 100 µL of dish soap was added. It was out for two hours in the dark and at room temperature. 570 nm was found to be the time of absorption.

The Optimal Cell Counts: When you trypsinize cells from the substrate, you scrape them from their surface. After that, put them back into the medium so that each m has 106 of them. It gradually decreases from 1 × 10^6 cells/mL to 1 × 10^3 cells/mL. Then, in three different wells on a microtiter plate, put 100 µL of each mix. For the absorption tests, leave three control wells empty so that they can be used as blanks. After that, the cells need to be kept in a jar for 6 to 48 hours at the optimal temperature and humidity for their specific type. Time is required for different types of cells, but for most, 12 hours to overnight is enough. Regardless of the well, you must add 10 microliters of MTT Reagent to it. Then, after two to four hours, return the plate to the liquid for cell growth. An upside-down camera was used periodically to check the cells and see if there was anything purple inside them. You should add 100 µL of Detergent Reagent to every well, even the test wells, once the purple stuff is clear enough to see under a microscope. Do not heat the cover or plate. For two to four hours or overnight, please refrain from touching it. Between 550 and 600 nm was the range of bands that the microtiter plate reader could read. It was used to measure the absorption in all wells, even the blanks, at 570 nm. You should use a group of cells that are in a straight line on the plot and have an absorption number between 0.75 and 1.25 for the test.

Spectrophotometer Test

One way to determine how much light a chemical can absorb is to shine a light through a sample solution and record the intensity of the light. It was performed using a Shimadzu UV-2401PC spectrophotometer equipped with an integrating sphere. It's made in Japan's Kyoto. It was also designed to organize things in a standardized way. A computer was connected to the spectrophotometer and used to save the spectral absorption plots of the mixes in the visible and UV ranges (190–1100 nm).

Statistical Analysis

Statistical analysis was performed on the collected data utilizing the SPSS statistical software (SPSS Version 22 for Windows; Chicago, IL, USA). The Kruskal–Wallis test ($p < 0.05$) was used to assess the inhibition zone sizes in millimeter and show the differences among the test mixtures (mixture one \ 3% Selenium Nano particles, mixture tow \ 4% Selenium Nano Particles, mixture three \ 5% Selenium Nano particles) with the control group SDF according to the antibacterial analysis for the three types of bacterial species. Post-hoc Bonferroni pairwise comparisons test with P-values were designed for multiple contrasts using Bonferroni correction.

Results

Antimicrobial Efficacy of SDF SeNps Mixture Preparations Against Isolated Strains

The potential antibacterial effect of the three mixtures and the control group was assessed using a modified agar diffusion technique, and the inhibition zone diameters were measured in millimeters. The following results were obtained.

In Table 1, the Kruskal-Wallis Test indicates there is a significant difference in the increasing diameter of inhibition zones among the test groups, including mixtures and control ($p = 0.01$), and the Post-hoc Bonferroni pairwise test shows that a significant difference appears between the SDF control group and mix 3 (5% Se).

Table 1: Median Range Of Inhibition Zones Diameters of SDF Plus Selenium Nano Particles Against *Enterococcus Faecalis*.

SDF Plus* Selenium Nps Against *Enterococcus							
Kruskal-Wallis Test				**Post-Hoc Bonferroni Pairwise Comparisons**			
Group	**N**	**Median (Range)**	**P* value**	**Sample 1 – Sample 2**	**Test Statistics**	**Std. Error**	**P value**
Entero plus mix1 (3% Se)	3	20 (2)	**0.01**	Entero plus mix 1 \ Entero plus SDF control	3.1	2.9	1.0
Entero plus mix 2 (4% Se)	3	24 (2)		Entero plus mix 1 \ Entero plus mix 2	- 2.6	2.9	1.0
Entero plus mix 3 (5% Se)	3	25 (0)		Entero plus mix 1 \ Entero plus mix 3	- 5.8	2.9	0.2
Entero Plus SDF control	3	16 (0)		Entero plus SDF control \ Entero Plus Mix 2	5.8	2.9	0.2
				Entero plus SDF control \ Entero Plus Mix 3	9.0	2.9	**0.01**
				Entero plus mix 2 \ Entero plus mix 3	- 3.1	2.9	1.0

In Table 2, the assessment of the inhibition zone diameters in millimeter reveals the Kruskal-Wallis Test, there is a significant difference of increasing the diameter of inhibition zones among the tests groups including mixtures and control (p = 0.02) and the Post-hoc Bonferroni pairwise shows that considerable difference appears between mix 1 (3% Se) and 3 (5% Se).

Table 2: Median Range of Inhibition Zones Diameters of SDF Plus Selenium Nano Particles Against *Lactobacillus*.

SDF Plus* Selenium Nps Against *Lactobacillus							
Kruskal-Wallis Test				**Post-hoc Bonferroni Pairwise Comparisons**			
Group	**N**	**Median (Range)**	**P* value**	**Sample 1 – Sample 2**	**Test Statistics**	**Std. Error**	**P value**
Lacto plus mix 1 (3% Se)	3	20 (1)	**0.02**	Lacto plus mix1\ Lacto plus SDF	- 3.0	2.9	1.0
Lacto plus mix 2 (4%Se)	3	25 (1)		Lacto plus mix 1\ Lacto plus mix2	- 6.0	2.9	0.2
Lacto plus mix 3 (5% Se)	3	28 (1)		Lacto plus mix 1\ Lacto plus mix 3	- 9.0	2.9	**0.01**
Lacto plus SDF control	3	22 (1)		Lacto plus SDF \ Lacto plus mix 2	3.0	2.9	1.0
				Lacto plus SDF\ Lacto plus mix 3	6.0	2.9	0.2
				Lacto plus mix 2 \ Lacto plus mix 3	- 3.0	2.9	1.0

In Table 3 the Kruskal-Wallis Test shows there is a significant difference of increasing the diameter of inhibition zones among the tests groups including the mixtures and control (p = 0.01) while the Post-hoc Bonferroni pairwise shows that significant difference appears between control SDF and mix 3 (5% Se). see Figure 1.

Table 3: Median Range of Inhibition Zones Diameters of SDF Plus Selenium Nano Particles Against *Streptococcus*.

SDF Plus* Selenium Nps Against *Streptococcus							
Kruskal-Wallis Test				**Post-hoc Bonferroni pairwise comparisons**			
Group	**N**	**Median (Range)**	**P* value**	**Sample 1 – Sample 2**	**Test Statistics**	**Std. Error**	**P value**
Strepto plus mix 1 (3% Se)	3	23 (1)	**0.01**	Strepto plus mix 1 \ Strepto plus SDF	3.0	2.9	1.0
Strepto plus mix 2 (4% Se)	3	24 (1)		Strepto plus mix 1\ Strepto plus mix 2	- 3.0	2.9	1.0
Strepto plus mix 3 (5% Se)	3	26 (2)		Strepto plus mix 1\ Strepto plus mix 3	- 6.0	2.9	0.2
Strepto plus SDF control	3	18 (1)		Strepto plus SDF\ Strepto plus mix 2	6.0	2.9	0.2
				Strepto plus SDF – Strepto plus mix 3	9.0	2.9	**0.01**
				Strepto plus mix 2 \ Strepto plus mix 3	- 3.0	2.9	1.0

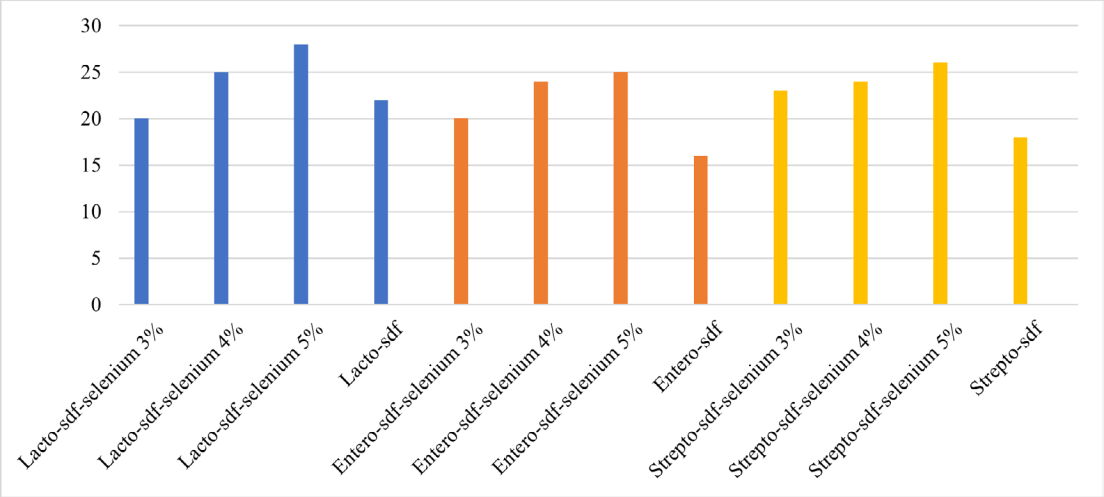


Figure 1: Inhibition Zones Diameters (mm) for Three Types of Bacteria According to four SDF Groups.

MTT Cytotoxicity Test

In an MTT assay, cytotoxic activity was assessed at 24, 48, and 72 hours. Within 24 hours, the results revealed that the SDF control IC50 was achieved at a dose of 39.53 mg/mL of material. While after adding Selenium nanoparticles within same time interval, the IC50 raise to 108.2 mg/ml, as showing in the Figure 2A below the cell viability percentage for SDF control showing that 46.54 % at a dose of 50mg/ml, while at the same dose of SDF mixed with Se Nps, cell viability was 79.15 % Figure 2B. Increasing the dose of MTT up to 400 mg/ml showing diminished decreasing of cell viability for both SDF control and SDF \Se mix (12.16 %, 19.23 %). Table 4 and Table 5 describe the cell viability and material doses increasing within the first 24 hours, showing the values of the SDF control and SDF mixed with 5% selenium, respectively.

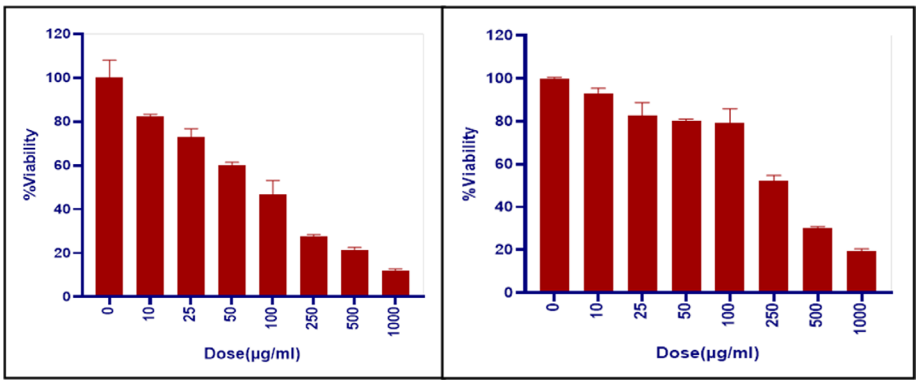


Figure 2: A, Cell Viability of SDF Control for 24hrs. B, Cell viability of SDF\Se mix for 24hrs.

Table 4: Mean of Cell Viability According to SDF Doses (µg/ml).

Dose(µg/ml)	N	Mean± SD
0	3	100 ± 8.05
6.25	3	82.42±0.91
12.5	3	73.07± 3.65
25	3	60.17±1.22
50	3	46.54±6.61
100	3	27.62±0.76
200	3	21.51±1.10
400	3	12.16±0.66

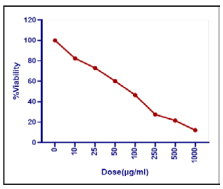
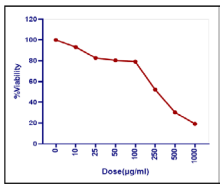


Table 5: mean of Cell Viability According to SDF + 5% SE Doses (µg/ml).

Dose(µg/ml)	N	Mean± SD
0	3	100±0.45
6.25	3	93.20±2.21
12.5	3	82.71±6.05
25	3	80.39±0.60
50	3	79.15±6.67
100	3	52.25±2.52
200	3	30.26±0.70
400	3	19.23±1.16

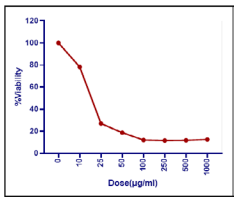


For 48 hrs. The IC50 of SDF control was 17.79 mg/ml, and for SDF\Selenium mix was 15.41 mg/ml, with a cell viability at 50 mg/ml dose (18.74%, 9.38%). Increasing the MTT dose up to 1000 mg/mL, the 12.52% cell viability of the SDF control decreased, and for the SDF-Se mix, it reached 9.97%, as shown in Figure 3.

Table 6 and Table 7 show mean values of SDF control and SDF mixed Nano concerning cell viability percent after 48 hours.

Table 6: Means of Cell Viability According to SDF Doses (µg/ml).

Dose(µg/ml)	N	Mean± SD
0	3	100±4.02
10	3	78.21±6.97
25	3	26.93±0.47
50	3	18.74±5.59
100	3	12.05±0.93
250	3	11.65±0.82
500	3	11.85±0.58
1000	3	12.52±0.23



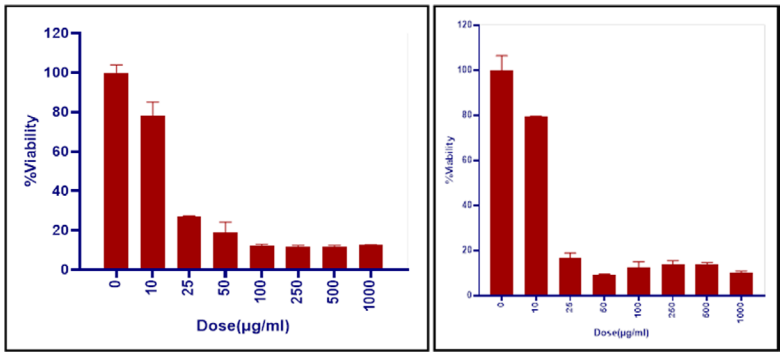
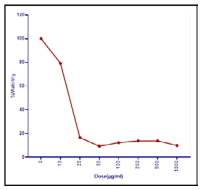


Figure 3: A: Cell Viability of SDF Control for 48hrs. B: Cell Viability of SDF\Se mix for 48 hrs.

Table 7: Mean of Cell Viability According to SDF + 5% SE Doses (µg/ml).

Dose(µg/ml)	N	Mean± SD
	3	100±6.42
10	3	79.22±0.44
25	3	16.62±2.22
50	3	9.38±0.09
100	3	12.34±2.55
250	3	13.79±1.59
500	3	13.82±0.74
1000	3	9.97±0.80



In 72 hours. The IC50 for SDF control and its mixture was (0.77, 1.91) mg/ml, respectively, and the cell viability at a 50 mg/ml dose for both was (8.31 %, 28.99 %). Increasing MTT up to 500 mg/mL shows the cell viability for both SDF control and SDF\Se mix decreased to (6.68%, 18.72 %), respectively. These results are clearly described in Figures 4A and Figure 4B and Table 8 and Table 9.

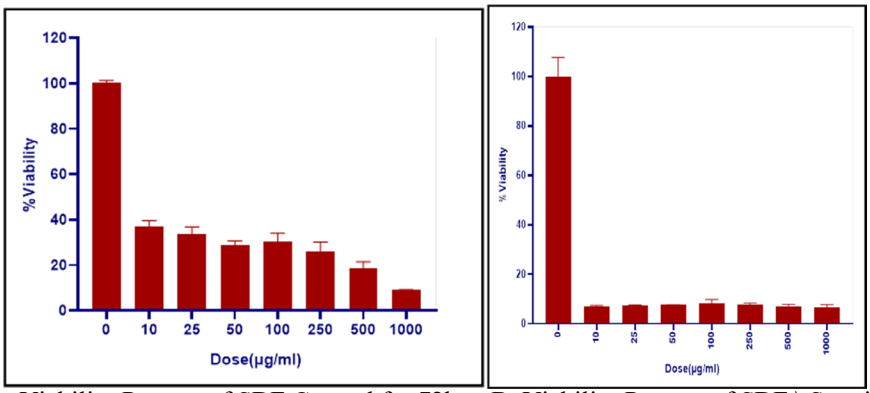


Figure 4: A, Viability Percent of SDF Control for 72hrs. B, Viability Percent of SDF \ Se mix of 72hrs.

Table 8 and Table 9 show mean values of SDF control and SDF mixed Nano concerning cell viability percent after 72 hours.

Table 8: Means of Cell Viability According to SDF Doses (µg/ml).

Dose(µg/ml)	N	Mean± SD
0	3	100±7.64
6.25	3	7.02±0.35
12.5	3	7.16±0.35
25	3	7.57±0.06
50	3	8.31±1.46
100	3	7.61±0.75
250	3	7.02±0.84
500	3	6.68±1.03

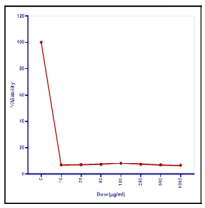
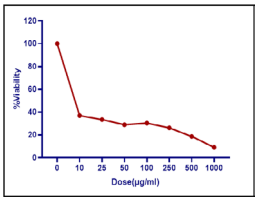


Table 9: Mean of Cell Viability According to SDF + 5% SE Doses (µg/ml).

Dose(µg/ml)	N	Mean± SD
0	3	100±1.43
10	3	36.96±2.56
25	3	33.46±3.29
50	3	28.99±1.66
100	3	30.39±3.67
250	3	26.28±3.90
500	3	18.72±2.78
1000	3	9.11±0.18



Spectrophotometer Analysis

In Table 10 below, it's noticeable that all the maximum absorption amount AU of all the experimental groups (SDF, SDF plus 3% selenium, plus 4% selenium, and 5% selenium) fall within the red or far-red region. This indicates that there is no change in the rational color of SDF after mixing with different ratios of selenium nanoparticles. However, no abnormal absorption was recorded that could affect the color of the original dental material, SDF.

Table 10: Shows the Minimum and Maximum Values of Absorption AU Within the Related Wavelength Spectrum in nm.

	SDF Only	SDF Plus 3% Selenium	Plus 4% Selenium	Plus 5% Selenium
Min in UV	0.32 AU at 362nm	0.30 AU at 244nm	0.44 AU at 262nm	0.26 AU at 248nm
Max in UV	3.61AU at 192nm	1.83AU at 190nm	4.74 AU at 220nm	2.48 AU at 192nm
Min in visible	0.32AU at 382nm	0.32 AU at 382nm	0.46 AU at 384nm	0.28 AU at 418nm
Max is visible	0.37 AU at 766nm	0.36 AU at 776nm	0.52 AU at 780nm	0.32 AU at 780nm

In Figure (5\A, B, C, and D) below, the scanning from VIS going through UV shows a regular pattern of wavelength absorbance in nanometers with close values, except for an intense abnormal value for SDF \ 4% Se mix of 0.529 at 780 nm within the visible range.

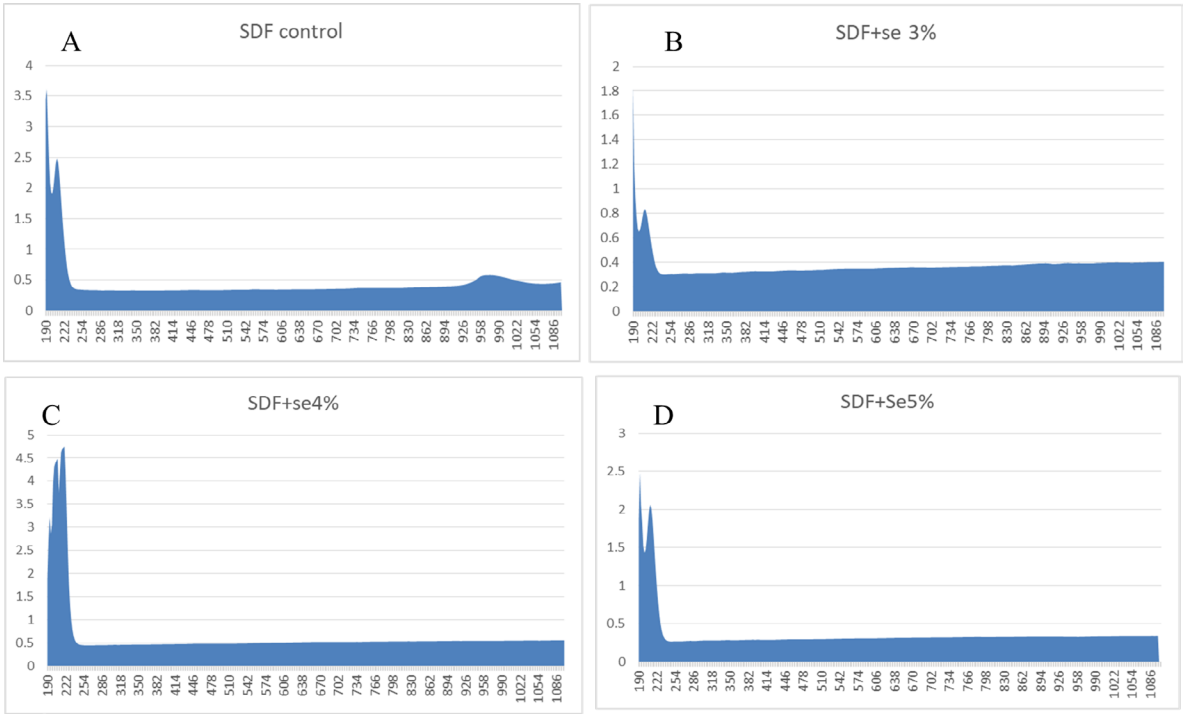


Figure 5: Wavelength Absorption in Nanometers in UV and VIS for: A, SDF Control, B, SDF plus 3% Se Nps, C, SDF plus 4% Se Nps, D, SDF plus 5% Se Nps.

Discussion

Microbiological Tests

There was a notable decrease of S. mutans on carious dentin surfaces in vitro following SDF treatment.[12] This is mostly due to the ability of varied silver fluoride forms to impede pathogen development.[13] In vitro tests demonstrate that inhibiting the growth of Lactobacilli produces equivalent favourable outcomes. This inhibitory action manifests in two primary ways: it impairs the enzymes that facilitate bacterial fermentation of sugars and carbohydrates, and it diminishes the probability of biofilm formation. These two methods are affected by 44,800 parts per million of fluoride ions.[14] Upon the application of SDF to dentin, the formation of silver phosphate and calcium fluoride crystals occurs as a result of the remineralisation process facilitated by these ions. This elevates the pH from 5.5 to 9–13. Calcium fluoride releases ions in acidic environments (cariogenic media), thereby storing these ions and functioning as an effective fluoride reservoir.[15] In the 1990s, Gotjamanos[16] proposed that silver ions might penetrate the pulp chamber via the dentinal tubules, possibly causing irritation to crucial odontoblast cells. Consequently, we must either safeguard the recipient's dentinal pulp area or identify a substitute for silver ions.[9,10] The applicability of SDF is limited; it requires enhancements to overcome certain deficiencies. One constraint pertains to the development of silver phosphate and silver sulphide, which contribute to its discolouration. When utilised in substantial amounts, these chemicals produce enduring black stains. Moreover, SDF significantly influences deep cavities; yet, it presents notable disadvantages, such as a metallic taste that may induce transient nausea or vomiting in some. This issue may be resolved by utilising reduced quantities of silver or a recovery agent that maintains or amplifies silver's anticarcinogenic attributes. Selenium nanoparticles represent an alternative form of antibiotic that may be utilised.

To ascertain if SDF can inhibit the progression of carious cavities, researchers examined the efficacy of SDF against the relevant bacteria at a concentration of 38%, utilising S. mutans and Lactobacillus as caries-inducing species.[17] Conversely, the rationale for selecting Enterococcus faecalis in our study, alongside the other two species, is its prevalence in infected root canals. Consequently, we aimed to evaluate the efficacy of silver diamine fluoride (SDF) as a potential alternative root canal disinfectant, which prompted us to explore additional applications of SDF and assess our findings regarding its effectiveness in this context.[18,19] In the agar diffusion assay, samples were maintained in contact with the three pathogenic bacteria for 18 hours. This duration would not be comparable to that of an endodontic procedure in a reputable dental practice, although it is sufficient for a dressing to prevent the formation of biofilms. The sizes of the inhibitory zones were quantified to evaluate the efficacy of the SDF nano-mixes, thereby replicating their clinical relevance.

The incorporation of Selenium Nanoparticles (SeNPs) into SDF markedly enhanced its efficacy in eradicating the three pathogenic species, contingent upon the percentage of nanoparticles added, which ranged from 3% to 5%, exhibiting a substantial difference. This discovery is

consistent with the research conducted by Han *et al.*[7], which shown that SeNPs can be utilised alongside or as a substitute for other pharmaceuticals to address multidrug-resistant microorganisms (MDR) when given topically, for instance, in patches.[20] Menazea *et al.*[8] also discovered that varying dosages of nanomaterials inhibited bacterial growth through diverse mechanisms. Various forms of selenium, including selenite and selenium nanoparticles, have demonstrated efficacy in eradicating infections in multiple investigations.[21,22] Those particles are excessively diminutive to enable their adherence to the surface of bacteria. This renders the cell wall fragile, facilitating the penetration of tiny Se ions. Reactive oxygen species (ROS) are produced in significant quantities intracellularly by these particles. This exacerbates oxidative stress. Reactive oxygen species (ROS) inhibit DNA damage and protein synthesis, hence obstructing cellular production and ultimately leading to cellular demise.[23,25] Certain pharmaceuticals appear to exhibit enhanced efficacy following the incorporation of SeNPs. The insertion of Se-NPs boosts many chemical properties, notably boosting the alkalinising effect associated with substantial changes in the preservation of pulp tissue.[25]

MTT Assays

Selenium is regarded as a trace element that is essential for human physiology. Nonetheless, a crucial distinction exists between therapeutic use within permissible limits and the dangerous, toxic amounts that are prohibited. Selenosis refers to selenium toxicity. The manifestations of this condition encompass gastrointestinal disturbances, baldness, tiredness, and irritability. Conversely, the prolonged dosages required for such an impact are significantly elevated, reaching up to 2400 µg/day.[26]

Elemental selenium is regarded as non-toxic in toxicity studies.[27] A significant decrease in toxicity risk is noted when examining the cytotoxicity of selenium nanoparticles.[28,29] The majority of these research concentrated on systemic in vivo effects, while only a limited number examined in vitro effects utilising mammalian fibroblast cells.[30]

The World Health Organisation recommends a daily selenium intake of 55 µg for healthy persons.[31] The cytotoxicity of selenium is due to its capacity to compromise cellular membranes and provoke oxidative stress.[26] Nevertheless, to alleviate these detrimental and toxic effects, stabilisation is feasible.[27] This example demonstrates how the polysaccharide mitigates the deleterious effects of selenium while simultaneously improving its bioavailability.[28]

This study also sought to determine if loading SDF with Se-NPs may exceed the cytotoxicity of SDF alone. The preliminary results of our investigation, after 24 hours, indicated a significant increase in cell viability. Cells treated with a combination of SDF and 5% selenium nanoparticles exhibit an extended lifespan compared to those treated just with SDF. The novel chemical formula of SDF additive combinations exhibits no acute cytotoxic activity. Selenium nanomaterials effectively mitigated the early cytotoxic responses of SDF. Based on this outcome, we can infer that the first diffusion of the mixed material will not detrimentally impact pulp vitality, hence fostering a healing environment. Nonetheless, clinical supporting studies and additional inquiry are essential to demonstrate that the combined Nano SDF is a highly biocompatible material.[29] No published research have been identified to evaluate the precise relationship to date. This outcome resembled that of Hassan *et al.*[14], who employed distinct nanoparticles with diverse sealer combinations. The fibroblast cell may exhibit a preference for proliferation on SDF infused with selenium nanoparticles. This corroborates the findings of Ramos and Webster[32], who determined that Se-NPs are biocompatible coating materials.

We detect a decline in fibroblast cell viability at 48 and 72 hours. Nanoparticles further augment the cytotoxicity of the SDF control.

Spectrophotometry Analysis

The colour changes of the SDF control were evaluated at 48 hours, comparing them with three mixtures. The spectrophotometer results indicated no differences in absorption values (AU) across visible and ultraviolet wavelength spectra. Contrary to the findings of Almulhim *et al.*[15], the incorporation of zinc nanoparticles (at varying concentrations) as a pre-treatment for dentin blocks significantly diminished the discolouration of dark lesions induced by silver diamine fluoride (SDF) in a concentration-dependent manner, offering a potential solution to a major aesthetic challenge associated with SDF applications. The discrepancy in our study results may stem from varying colour analysis methodologies or differing nanoparticle manufacturing methods, despite both being antioxidant reagents. Additional examination of the colour alteration in Se-NPs is required for their application to dentine blocks.

Conclusion

SeNPs demonstrate substantial anti-inflammatory characteristics alongside remarkable antibacterial efficacy, so greatly augmenting the features of SDF and rendering its Se mixes a promising anti-caries agent. Beyond their efficacy in arresting dentin caries, their application as a canal disinfectant could provide an additional advantage due to their antibacterial properties against the notoriously root canal-resistant E. faecalis species. The biocompatibility of these nanoparticles exhibits a favourable impact on the SDF material, enhancing cell survival in the presence of these particles relative to SDF alone. Regrettably, our experiment does not address the anticipated limitation of SDF discolouration, and the Spector assay results indicate a consistent, restricted range across both UV and VIS wavelengths.

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