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Investigating the effect of selenium nano-particles on microbial activity and cancerous cell line of MCF-7 and MDA-MB-231

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Abstract: Selenium is a mineral that is essential to human health and is widely recognized for its responsibilities as a powerful anticancer vitamin and antibacterial vitamin. Selenium also plays a critical part in the production of vitamin D. The purpose of this research was to evaluate the particular effects that selenium nano-particles (SeNPs') had on the infectious agent Staphylococcus aureus as well as the breast cancer cell lines MCF-7 and MDA-MB-231. The proportion of MDA-MB-231 and MCF-7 cells that underwent late apoptosis was dramatically increased by selenium nanoparticles, whereas the number of cells that underwent cell expansion was significantly reduced. There was a wide range of variability in the effects of selenium nanoparticle treatment on cell growth apoptosis, apoptosis rates and patterns of cell cycle arrest. After 2, 4 and 6 hours, researchers found that the development of S. aureus was significantly reduced by selenium nanoparticles at doses of 8.0, 16.0 and 32g/mL. In addition to this, the presence of selenium nanoparticles resulted in a reduced percentage of bacteria that were still alive. According to the findings of the study, there is a need for more research into selenium nanoparticles with the intention of preventing and treating infections caused by S. aureus.

Keywords: Selenium nanoparticle, cell line MCF-7, proliferation.

INTRODUCTION

In recent years, research into selenium nanoparticles (also known as SeNPs) has taken on an increasingly important role as a result of the potential relevance it has for a wide variety of physiological functions. When compared to selenium, selenium nanoparticles result in greater absorption when used in conjunction with regular dosing. In order to increase the transportability, bioavailability, and bioactivity of selenium compounds (selenoproteins, selenoenzymes, and the like), novel techniques are required. SeNPs have garnered a lot of interest from the scientific community due to the fact that there is a possibility that they might be used as therapeutic agents and food additives. Due to the antioxidant, antibacterial, anticancer and anti-diabetic characteristics of selenium, the incorporation of selenium nanoparticles into biomedical and pharmaceutical research is a realistic possibility. With the assistance of selenium nanoparticles, it is possible to circumvent the presence of pollutants and heavy metals. Since SeNPs are able to absorb metals and heavy metals, it is possible that they might be beneficial for the process of decontaminating polluted water and soil. It has been proposed that sodium selenate, sodium selenite, selenium dioxide, and selenium tetrachloride may be converted into selenium nanoparticles by the process of bioreduction (Al-Otaibi *et al*., 2022).

In women, breast cancer is the most frequent form of invasive cancer and it is also the second leading cause of death. When breast cancer has progressed to an advanced stage, surgery is often followed by a course of chemotherapy, immunotherapy, radiation therapy, or any combination of these treatments. According to research conducted by Sun *et al*. (2017), metastatic breast cancer is the most dangerous and incurable form of the disease. The

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median overall survival time for patients with this kind of the disease is just two to three years.

Selenium is a mineral that is essential to human health and is widely recognized for its responsibilities as a powerful anticancer vitamin and antibacterial vitamin. Selenium also plays a critical part in the production of vitamin D. Consumption of selenium has been demonstrated in a number of ecological and case-control studies to be negatively linked to the risk of breast cancer as well as the death rate associated with the disease (Zhang *et al*., 2013). Studies conducted in vitro utilizing breast cancer cells revealed that treatment with selenium decreased the proliferation of the cancerous cells. When administered at a dose of 0.8 milligrams per kilogram of body weight, selenium reduced the growth of breast cancer cells by an amount ranging from 80-93%. Because of the natural cell damage that is caused by cancer therapies like chemotherapy and radiation therapy, lymphedema may become permanent in certain cancer patients. This is particularly true for individuals who have had radiation therapy. Selenium is a mineral that has lately been suggested as a potential alternative treatment for lymphedema brought on by radiation therapy and chemotherapy. Taking selenium supplements may be advantageous for individuals with advanced breast cancer because of selenium's anticancer characteristics and its ability to reduce the negative effects of chemotherapy, including lymphedema. However, it is yet unknown how the combination of chemotherapy and selenium would affect advanced breast cancer (Liu *et al*., 2016). Numerous pieces of research from the scientific community have shown that selenium is an excellent weapon against microorganisms. Selenium-rich environments have been shown to greatly reduce the growth of pathogenic Escherichia coli probiotics in both animal and laboratory settings. This has been shown via several scientific studies. A dose of 0.509g selenium per gram of probiotics was shown to suppress the development of E. coli after 96 hours in vitro. This was proved by the fact that the growth of E. coli was slowed down. Mice were given probiotics that were either rich or low in selenium *in vivo* for a period of 28 days before being injected with E. coli. In the group that was treated, the number of deaths that occurred was much lower. Organoselenium compounds such as 2,4,6-tri-para-methoxy phenyl selenopyrylium chloride, 9-para-chlorophenyl octahydro selena xanthene and perhydroselenoxanthene were synthesized and shown to have an antibacterial action in vitro. This effect was found to be especially effective against *S. aureus*. On the other hand, very little is known about the effect that elemental selenium nanoparticles have on bacteria. The bacteria known as staphylococcus aureus is a significant one that has been linked to a broad variety of diseases. Infections caused by *S. aureus* may be difficult to treat because the bacteria can form biofilms and are resistant to medicines. Despite the fact that selenium has been employed for a variety of reasons, including as a medicine to treat cancer, the effects of selenium on microbes are not currently the subject of extensive research at this time. In this work, we investigated the effect that selenium nanoparticles (SeNPs) had on S. aureus, in addition to the breast cancer cell lines MCF-7 and MDA-MB-231.

MATERIALS AND METHODS

Preparation of selenium nano-particles

The production of selenium nanoparticles began with the reduction of sodium selenite with glutathione, which was followed by the stabilization of the process using bovine serum albumin (BSA). We combined 0.15 g of BSA with 9mL of double-distilled water, 3mL of 25mM Na2SeO3, 3mL of 100mM GSH and 3mL of double-distilled water in a sterile cabinet. The final mixture included 100mM GSH. All of the solutions were sterilized using double-distilled water, which was utilized in an atmosphere that was kept sterile. The pH of the reactant solution was brought into the alkaline range by adding 1M of NaOH after it had been well mixed. The addition of NaOH led the white reactant solution to become red, which is a sign that selenium nanoparticles were formed. Before being used in bacterial research, the selenium nanoparticles were separated using centrifugation at 13,000 rpm, disinfected using ultraviolet light irradiation and resuspended in sterile double-distilled water five times. This was done in order to ensure that the nanoparticles were free of any bacteria. Both the National Institute of Agriculture and Biotechnology in Faisalabad and Government College University in Faisalabad, which both offer facilities that are on the leading edge, played host to the research that was carried out (Wang *et al*., 2019).

An investigation of the effects of SeNPs on a breast cancer cell line

Cell culture preparation

The University of Health Sciences in Lahore provided us with the MDA-MB-231 and MCF-7 human breast cancer cell lines to use in our research. On tissue culture plates, the test cells were cultured in fetal bovine serum (FBS) supplemented DMEM at a concentration of 10%. (5*100000) (Gomathi *et al*., 2020) The cells were maintained in an incubator that was humidified at 37 degrees Celsius and contained 5% carbon dioxide.

Following treatment of each cell line with SeNPs and subsequent seeding of 5100,000 cells in DMEM containing 10% fetal bovine serum, growth was evaluated and compared. After a period of 24 hours in which the cells were allowed to proliferate in fresh medium, they were washed with phosphate-buffered saline. In order to verify the effects of selenium on cell growth and survival, MDA-MB-231 and MCF-7 cells were given 8, 16, or 32g/mL of selenium nanoparticles and treated for a period

of 72 hours. The cells were stained with trypan blue at a concentration of 0.38 percent and then counted using a hemocytometer. At least three independent runs of each experiment were carried out and measurements were collected in both directions twice for each variable.

Checks for the death of apoptotic cells

It was advised by the manufacturer that the annexin V stain be used, thus that is what was done. Trypsin and ethylenediaminetetraacetic acid were used in the process of developing single-cell suspensions. After the cell lines were treated, they were centrifuged twice with cold PBS and then they were resuspended in a binding buffer that included 10mM HEPES, pH 7.4, 150mM NaCl, 5mM KCl, 1mM MgCl2 and 1.8mM CaCl2. An aliquot (100 l) of the solution that included 1 105 cells, 5 l of Annexin V-fluorescein isothiocyanate (FITC) and 5 l of propidium iodide (PI) was added to each culture tube that was 5mL in volume and then refilled. Following an incubation period of 15 minutes in the dark at a temperature of 25 degrees Celsius, 400mL of the binding buffer was added to each tube and the contents of each tube were then vortexed. Flow cytometry was performed on a sample with the help of FACSCalibur instruments in a little over half an hour (Ezhilarasan *et al*., 2019).

Antimicrobial potential of selenium nano-particles

Bacterial culture preparation

Lyophilized bacterial cells from the University of Karachi's Microbiology Department were used to buy a cell line that produces biofilm from the bacterium *S. aureus.* The cells were cultured in a tryptic soy broth (TSB) concentration of 30 mg/ml. After the bacteria had developed to the point where they were able to enter the stationary phase, they were flash-frozen in a solution that consisted of glycerol and TSB in equal parts. In each of the experiments, the frozen supply was used. The day before the injection, the bacteria for the bacterial injection were removed from the frozen stock using a sterile 101 loop. This was done in order to maintain the integrity of the bacteria. After spreading the germs out over TSB agar, we let them 37 degrees Celsius and 16 hours to develop before observing the results. After cultivating the bacteria in a sterile loop for 24 hours, three milliliters of TSB were injected into a test tube. This was done after the bacteria had been cultured. We circulated the tube at a rate of 275 revolutions per minute and placed it in an incubator set to 37.1 degrees Celsius in order to stimulate the bacteria into entering the exponential growth phase. We were able to calculate the concentration of the bacteria in the solution by first measuring the optical density of the solution at 561 nm and then extrapolating those data with the use of a standard curve that showed the proportionality between the optical density and the concentration. On the next page, you will find a description of experiments that were conducted using a bacterial solution that included 30,000 microorganisms per milliliter.

Antimicrobial evaluations currently being conducted

We investigated how the development of S. aureus was affected by exposure to selenium nanoparticles at concentrations of 8, 16 and 32g/ml. After infusing bacterial solutions with selenium nanoparticles and cultivating them at 37.1% temperature, 275 rpm shaking, and 45% humidity, the bacterial solutions were cultivated for 2, 4 and 6 hours. In the absence of selenium nanoparticles, bacteria were grown in TSB at 37 degrees Celsius, 95% humidity, 5% carbon dioxide and 250 revolutions per minute. When TSB that had been cleaned of all microorganisms was used to make blank solutions, the quantities of selenium nanoparticles that were specified before were added to the mixture. For the controls, we utilized TSB solutions that were sterile and free of selenium and bacteria. After incubation, the bacterial concentration of the solution was determined by comparing the solution's final optical density to a reference curve and graphing the results. After adding 200 mL of bacterial solution, control solution, or blank to each well of a 96-well plate and reading the plate with a SpectraMax M5 plate reader, the optical densities were measured at 562 nm. The measurements were taken. We found that there were significant differences in the optical densities of the bacterial solutions compared to the values of the blanks.

The microbe's status as viable or nonviable

After the allotted amount of time had passed, we used the Bac Light Bacterial Viability Kit to conduct live/dead animal experiments in accordance with the instructions provided by the manufacturer. In order to conduct the analysis of the fluorescent signals, a Molecular Devices Spectra Max M5 fluorescence micro plate reader was used.

STATISTICAL ANALYSIS

The data were gathered from at least three different tests in total. Student t-tests were used to make the comparison between the findings obtained from the experimental cells and the outcomes of the control cells. In order to assess the continuous variables that followed a normal distribution, one-way analysis of variance tests were carried out. When comparing continuous variables with non normal distributions, Kruskal-Wallis tests were used as the statistical method of choice. Chi-square tests were used in order to make comparisons between the nominal variables. The efficiency of the antibacterial treatment was evaluated three times with duplicate samples each time. After gathering all of the data, we used a Student's t-test using only one direction of analysis to seek for statistically significant deviations. At every stage of the statistical analysis process, Microsoft Excel (Redmond, WA) was used. For this analysis of the data, we utilized SPSS 18.00. We considered p-values with two-tailed significance tests that were lower than 0.05 to be statistically significant.

RESULTS

Antimicrobial potential of selenium nano-particles on bacterial culture

The introduction of selenium nanoparticles to the bacterial solution reduced growth after 2 hours (and continued to do so for another 4 and 6 hours). Fig. 1 provides a more detailed depiction of the bacterial growth profile after exposure to selenium nanoparticles. Ignoring the correlation between variables. fig. 1 illustrates that bacterial growth is slowed and inhibited when selenium nanoparticles are present. Bacterial growth was reduced by factors of 20 after 2 hours, 23 after 3 hours and 30 after 6 hours when compared to the controls.

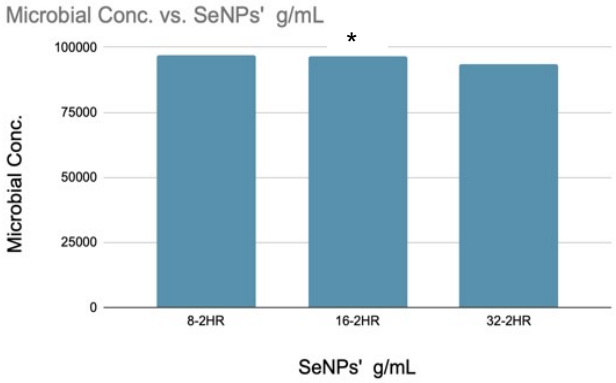

Fig. 1: Microbial assay

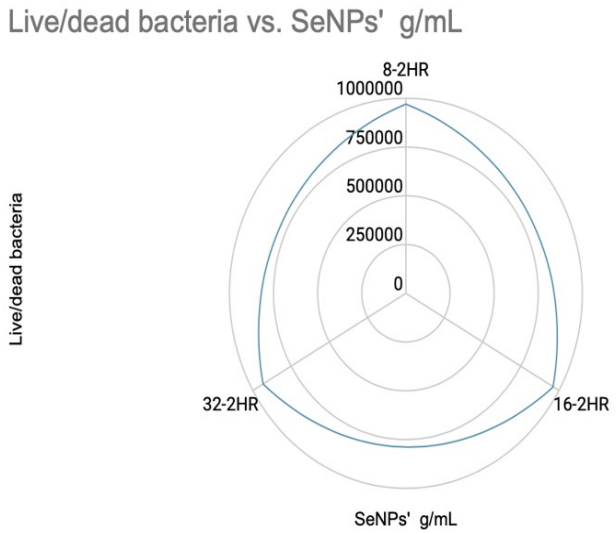

Fig. 2: Live/dead bacteria

Live/Dead microbes

The bacterial viability of each solution was further evaluated using live/dead assays. The findings showed a significant decrease in the percentage of live bacteria in the treated solution across all three time periods examined (2, 4 and 6 hours). Sixty percent of the bacteria were successful in the experiments without selenium nanoparticles. Three different concentrations of selenium nanoparticles were evaluated, and there was no discernible difference in the percentage of alive cells between any of them. At all of the selenium nanoparticle concentrations and time points considered, there was no discernible change in the percentage of alive cells. The live/dead findings demonstrated that the bacteria were really destroyed by the selenium nanoparticles, as opposed to only having their development slowed (fig. 2).

Cancer cell growth

A and B show the results of treating the MDA-MB-231 and MCF-7 cell lines with 8, 16 and 32g/mL of selenium nanoparticles, respectively. Cells were stained with trypan blue and counted in a hemocytometer to establish their viability. After being exposed to the drug at several doses, the graph displays the percentage of the indicated cells that have survived. These figs. are the mean and standard deviation of at least three separate studies (fig. 3)

Apoptosis analyses

DNA content was shown to be reduced at selenium nanoparticle concentrations of 8.0, 16.0 and 32g/mL. The biggest benefits, as seen in fig. 4, were seen at the highest concentration of selenium nanoparticles.

DISCUSSION

According to the results of the research, selenium nanoparticles reduced the development of Staphylococcus aureus at concentrations of 8, 16 and 32g/mL for the course of the 2, 4 and 6 hour time periods that were investigated (fig. 1). The present investigation demonstrated equivalent results to those observed in previous studies on the antibacterial effectiveness of SeNPs; however, the reported value for these findings was only 30%. Researchers at the University of California, Santa Cruz used an innovative method of colloidal synthesis to manufacture selenium nanoparticles. These nanoparticles greatly inhibited the development of S. aureus compared to the control group, which received no therapy. The early suppression of Staphylococcus aureus by selenium nanoparticles (up to 5 hours early) has the potential to prevent the development of biofilms. Experiments comparing live and dead bacteria revealed that after 3, 4 and 5 hours of exposure to selenium nanoparticles, only forty percent of S. aureus were destroyed. It is necessary to conduct more study and development of these potential antibacterial nanoparticles in order to shed light on the processes that are responsible for the antibacterial properties of selenium. According to Tran, P.A. and Webster, T.J.'s (2011) research, sodium selenite should be utilized as a positive control, and silver nanoparticles should be used as comparisons.

Using live/dead tests, additional investigation of the bacterial viability of each solution was carried out. The findings demonstrated that the amount of live bacteria in

the fluid that included selenium nanoparticles was significantly reduced during the course of the whole experiment, regardless of the dosage of selenium nanoparticles that was being examined or the length of time that the experiment was being conducted. Klebsiella sp. was shown to be the most susceptible to the antibacterial effects of the optimized nanoparticles in a second investigation that compared the effectiveness of the optimized nanoparticles against cultures of Gram-positive and Gram-negative bacteria. The use of biosynthesized and optimized selenium nanoparticles in pharmacological and industrial applications is growing (Menon *et al*., 2020).

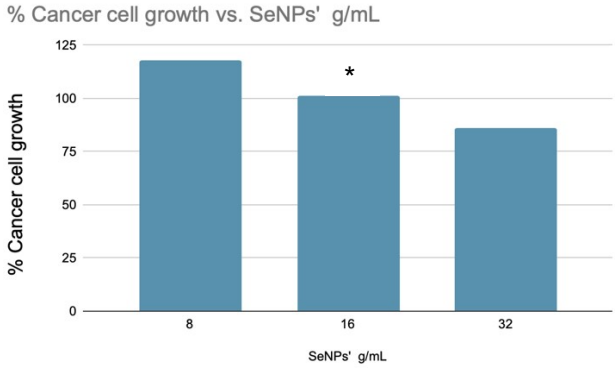

Fig. 3: Cancer Cell Growth
*P-value below 0.05.

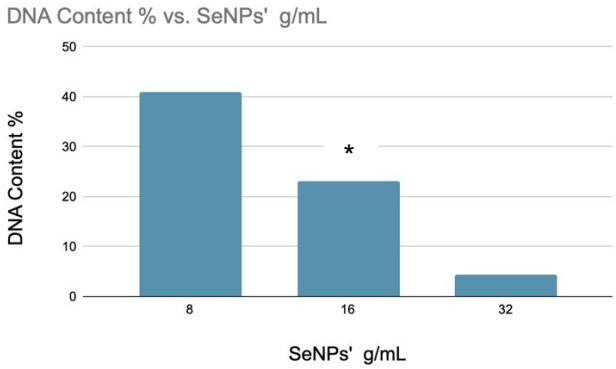

Fig. 4: DNA content
*P-value below 0.05

The outcomes of treating the MDA-MB-231 and MCF-7 cell lines with 8, 16, and 32g/mL of selenium nanoparticles, respectively, are shown in both A and B. In order to determine whether or not the cells were still alive, they were stained with trypan blue and then counted using a hemocytometer. The survival rate of the cells that were indicated is shown as a percentage on the graph after they were subjected to the medicine in a variety of dosages. According to the findings of the research, growth rates decreased down. The researchers who were investigating the anticancer potential of selenium combined therapy in MDA-MB-231 cells found that the combination therapy group had significantly reduced cell growth (docetaxel at 500pM plus selenium at 10M; P = 0.004), increased late apoptosis (63% vs. 26%; P 0.001), and cell cycle arrest at G2/M (P 0.001) compared to the control group.

After continuous exposure to the electromagnetic radiation (EMR) released by mobile phones, the authors of the research found that free oxygen radical generation, apoptosis and mitochondrial depolarization may all rise. This research attempted to evaluate how the antioxidant redox system, mitochondrial apoptosis, and cell death were impacted by 900 MHz radiation. The MDA-MB-231 breast cancer cell line was used for the investigation. A comparison was made between the primary cancer cell cultures and those of the selenium group, the EMR group and the EMR plus selenium group. For one hour, cells in the EMR group were exposed to electromagnetic radiation at a frequency of 900 MHz, with a specific absorption rate (SAR) value of 0.36 W/kg. The cells in the selenium groups were treated with sodium selenite for an extra hour before they were exposed to electromagnetic radiation (EMR). After that, measurements were taken of cell viability, the production of intracellular reactive oxygen species (ROS), the depolarization of the mitochondrial membrane, apoptosis, and the levels of caspase-3 and -9. According to the results of the MTT test, a decrease in oxidative stress and an increase in the potential of the mitochondrial membrane offered protection for the cells against the oxidative damage caused by EMR. The anti-apoptotic effects of selenium were shown by monitoring the amounts of caspase-3 and caspase-9 in the apoptotic process. A study in which apoptosis was induced by selenium nanoparticles came to the conclusion that, while selenium incubation appeared to prevent effects on apoptosis and oxidative stress, 900 MHz EMR appeared to promote apoptotic effects via oxidative stress and mitochondrial depolarization (Kahya *et al*., 2014). The study was published in the journal Kahya, M.C.

CONCLUSION

Use of selenium nano-particles not only possesses antimicrobial activities but also showed strong anticancer potential in the MDA-MB-231 and MCF-7 cells. SeNPs' not only reduced bacterial growth but also reduced live bacteria count. It was found useful in reducing the cancer cell proliferation and growth.

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