

EFFECT OF NANOSELENIUM ON FISH GUT BACTERIA

Submitted in partial fulfillment of the requirements for the award of
Bachelor of Science Degree in Biotechnology

By

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**DEPARTMENT OF BIOTECHNOLOGY
SCHOOL OF BIO & CHEMICAL ENGINEERING**

SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
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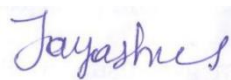
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DEPARTMENT OF BIOTECHNOLOGY

BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **Sharon.S (40770048)**, who carried out the project entitled "**Effect of Nanoselenium on fish gut bacteria**" under our supervision from **November 2022 to April 2023**.


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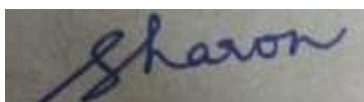
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DECLARATION

I **Sharon. S**, hereby declare that the Project Report entitled “**Effect of Nanoselenium on fish gut bacteria**” done by me under the guidance of **Dr. S. Jayashree** (Internal Guide) and **Dr. T. Stalin Dhas** (External Guide) at **Sathyabama Institute of Science and Technology, Chennai, Tamilnadu, India**, is submitted in partial fulfillment of the requirements for the award of **Bachelor of Science Degree in Biotechnology**.

A rectangular box containing a handwritten signature in blue ink that reads "Sharon".

12/05/2023

SIGNATURE OF THE CANDIDATE

CHENNAI

ACKNOWLEDGEMENT

I am pleased to acknowledge my sincere thanks to Board of Management of **SATHYABAMA** for their kind encouragement in doing this project and for completing it successfully. I am grateful to them.

I convey my thanks to **Dr. S. Jayashree**, Department of Biotechnology, for providing me necessary support and details at the right time during the progressive reviews.

I would like to express my sincere and deep sense of gratitude to my Internal Guide **Dr. S. Jayashree**, Department of Biotechnology and my External Guide **Dr. T. Stalin Dhas**, Assistant Professor/Scientist-B, Centre for Ocean Research, Col. Dr. Jeppiaar Research Park. I would also like to thank **Dr. D. Inbakandan**, Head of Centre for Ocean Research and research scholar **Ms. P. Sowmiya** and **Mr. S. Lakshmi Narayana**, Centre for Ocean Research for their valuable guidance, suggestions and constant encouragement paved way for the successful completion of my project work.

I wish to express my thanks to all Teaching and Non-teaching staff members of the Department of Biotechnology, who were helpful in many ways for the completion of the project.

ABSTRACT

Selenium is an essential micronutrient for most aquatic organisms, but elevated selenium exposure can cause adverse effects in fish, with a narrow range between essential and toxic levels. Nanoselenium has attracted growing attention due to its high availability, low toxicity and its possible applications in the prevention of oxidative damage in animal tissues. Nanoparticle toxicity is the ability to organize around nano particles protein concentration that depends on particle size, curvature, shape and surface characterization charge. The toxicity of selenium nanoparticle (SeNPs) in the laboratory by conducting 96 h toxicity experiments. The CS-SeNPs were synthesized by reducing sodium selenite using ascorbic acid and characterized by spectroscopic and microscopic techniques. UV-visible spectroscopic has a distinctive peak of 260 nm which indicates the selenium and absorbance and other techniques to find the stability, functional groups and morphology of selenium. Some toxicity test like cytotoxicity, lipid peroxidation and Reactive oxygen species (ROS) estimation are used to analyze the microbes toxicity level by exposure of CS-SeNPs to bacteria. Selenium salts are toxic in large amounts, but trace amounts are necessary for cellular function in many organism.

KEYWORDS: Bioavailability, Selenium nanoparticles, Toxicity analysis.

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LIST OF ABBREVIATION

CS-SeNPs	Chitosan Selenium Nanoparticle
DCFH-DA	2,7- Dichlorofluorescein diacetate
DAPI	4,'6-diamidino-2-phenylindole
ELISA	Enzyme Linked Immunosorbent Assay
FTIR	Fourier Transform Infrared
g	gram
hr	hour
mL	Milliliter
µg	Microgram
MTT	3 - (4,5-dimethylthiazol-2-yl)-2-5-diphenyl tetrazolium bromide
Na₂SeO₃	Sodium Selenite
PSA	Particle size analyzer
ROS	Reactive Oxygen Species
rpm	Revolution per minute

Se

Selenium

TEM

Transmission Electron Microscope

UV-VIS

Ultraviolet - Visible Spectroscopy

CHAPTER 1

1. INTRODUCTION:

Selenium nanoparticles are nanoscale particles composed of selenium atoms. They have gained considerable attention in recent years due to their potential biomedical applications, including antioxidant, antimicrobial, anticancer, and immunomodulatory effects. The nanoparticles were characterized using UV-Vis spectroscopy, transmission electron microscopy, X-ray diffraction, Fourier transform infrared spectroscopy and particle size analysis. Selenium is well known for its photoelectrical and semiconducting properties therefore it is used in solar cells, rectifiers, photographic exposure meters, and xerography (Johnson et al., 1999). The nanoform of selenium is all-important for the research due to its high degree of absorption and low toxicity than its organic and inorganic forms (Bhattacharjee et al., 2019).

Selenium nanoparticle has been regarded as a promising material for many applications due to their unique properties like high biological activity, bioavailability, low toxicity, high particle dispersion, and large surface area (Chen et al., 2008; Torres et al., 2012). Selenium nanoparticles are particles composed of selenium atoms with dimensions ranging from 1-100 nm. It can be synthesized through various methods including chemical reduction, microbial reduction, and photochemical synthesis. These methods allow for control over the size, shape, and properties of the nanoparticles which can be useful in a variety of applications. The biologically synthesized selenium nanoparticles (SeNPs) are stable, as they do not aggregate due to the capping and stabilization by the biomolecules on their surface. Selenium occurs in multiple oxidation states of +6, +4, 0, and -2 (Bajaj and Winter, 2014; Eiche et al., 2015; Jain et al., 2015a; Navarro-Alarcon and Cabrera-Vique, 2008; Shakibaie et al., 2015). Selenium in the form of selenate oxyanion (SeO_4^{2-}) is more toxic than the selenite oxyanions (SeO_3^{2-}). Elemental nano selenium (Se_0) is insoluble and less toxic (Wadhvani et al., 2016; Zhang et al., 2005).

Nanoscale materials, including nanoparticles of selenium (Se), have gained significant attention in recent years due to their potential applications in various fields, including medicine, environmental remediation, and agriculture. Selenium is an essential micronutrient for many organisms, including fish, and plays a vital role in various physiological processes. The effects of nanoscale selenium on fish gut bacteria, which play a critical role in the health and well-being of fish, are not well understood. Intestinal microbiota in fish are known to be highly diverse and play important roles in maintaining host health, including nutrient metabolism, immune modulation, and protection against pathogens (Ringo and Gatesoupe, 1998; Nayak, 2010). Therefore, it is essential to understand the potential impacts of nanoscale selenium on the gut microbiota of fish.

Nanoselenium is a form of selenium with particle sizes ranging from 1-100 nm, which has gained attention due to its potential application in aquaculture as a dietary supplement for fish. Nanoselenium (SeNPs) has gained significant attention in recent years due to its potential application in different fields, including medicine, agriculture, and environmental science. As a result, the use of nanoselenium has increased in aquaculture practices to improve fish health and growth. The effect of nanoselenium on fish gut bacteria is still not well understood. It has gained attention due to its potential as a highly bioavailable and effective form of selenium supplementation for aquatic organisms, including fish. Selenium is an essential micronutrient for fish, but its bioavailability can be limited in aquatic environments. Nano-Se has been shown to improve the growth and immune responses of fish, as well as reduce the accumulation of toxic metals in their tissues (Zhang et al., 2021). The gut microbiota plays a crucial role in fish health, including nutrient absorption, immune function, and disease resistance. Recent studies have shown that selenium supplementation can alter the gut microbiota in animals, including mice and chickens (Yang et al., 2020; Yang et al., 2021). It is possible that Nano-Se supplementation could also impact the composition and function of fish gut microbiota. Therefore, further research is needed to

investigate the effects of Nano-Se on fish gut microbiota and the potential implications for fish health and aquaculture practices.

Nanoscale materials are being widely used in various fields due to their unique properties and potential applications. Selenium is an essential micronutrient for humans and animals, but its deficiency can cause various diseases. Nanoscale selenium has been reported to have better bioavailability and efficacy compared to other forms of selenium. Therefore, NanoSe is being explored as a potential alternative to traditional selenium sources. The potential impacts of NanoSe on the gut microbiota of aquatic organisms, such as fish. The gut microbiota plays an important role in the health and well-being of fish, and any disruption to the microbial balance can have detrimental effects. These findings suggest that NanoSe may have a positive effect on the gut microbiota of fish, which could have potential implications for the health and performance of aquatic organisms. Further research is needed to fully understand the mechanisms underlying these effects and to determine the optimal dose and duration of NanoSe supplementation for fish.

Selenium nanoparticles consists of three methods:

- **Physical method** : Pulsed laser ablation, UV irradiation, Gamma irradiation, Microwave irradiation, Sonochemical methods and Ultrasonic treatment (Quintana et al., 2002).
- **Chemical method** : Wet chemical method, Solvothermal process, Solution phase technique and Riley oxidation (Gao et al., 2003).
- **Biological method** : Plant extracts (lemon leaf, fenugreek seed, etc..) and microorganisms (bacteria, algae, yeast and fungi) are used for reduction (Prasad et al., 2013).

Role Of Selenium:

- Reduce oxidative stress.
- Active centre for many enzymes.
- Maintain function of thyroid, pancreas, reproductive system, immune system.
- DNA repair.
- Detoxification.
- Cell cycle maintenance.

Applications of Selenium Nanoparticle:

Selenium nanoparticles (SeNPs) have a wide range of potential applications due to their unique properties and mechanisms of action. Here are some of the main areas where SeNPs are being studied and applied:

- **Antioxidant therapy:** SeNPs have strong antioxidant properties, which makes them promising candidates for treating diseases caused by oxidative stress, such as neurodegenerative disorders, cardiovascular diseases, and cancer. SeNPs can scavenge free radicals and protect cells from oxidative damage, and have been shown to be effective in animal models of these diseases (Liu et al., 2018).
- **Cancer therapy:** SeNPs have been shown to have anticancer properties, and are being studied as potential treatments for various types of cancer. SeNPs can induce apoptosis (programmed cell death) in cancer cells, and can also modulate the immune system to enhance the body's ability to fight cancer (Wang et al., 2014).
- **Drug delivery:** SeNPs can be used as drug delivery vehicles, due to their unique surface properties and ability to target specific cells or tissues. SeNPs can be loaded with drugs or other therapeutic agents,

and can be designed to release their cargo in a controlled manner (Li et al., 2015).

- **Imaging:** SeNPs can also be used as contrast agents for imaging techniques such as computed tomography (CT) and magnetic resonance imaging (MRI). SeNPs can enhance the visibility of tissues and organs, and can be designed to target specific areas for more accurate imaging (Zhang et al., 2017).
- **Food and agriculture:** SeNPs have potential applications in food and agriculture, including as a food supplement or feed additive for livestock to enhance immune function and prevent disease, and as a fertilizer to improve crop yields and resistance to stressors like drought and disease (Zhang et al., 2017).

The unique properties and mechanisms of action of SeNPs make them promising candidates for a wide range of applications in biomedical, imaging, and agricultural fields. The antioxidant, anticancer, and immune-modulating properties of SeNPs make them promising candidates for a variety of biomedical applications.

CHAPTER 2

2. REVIEW OF LITERATURE:

Nanoscale selenium (SeNPs) has been reported to possess a wide range of biological activities, including antioxidant, anti-inflammatory, and antimicrobial properties (Wang et al., 2016). In recent years, there has been growing interest in the potential use of SeNPs as a feed supplement for aquatic animals, including fish, due to their beneficial effects on growth, health, and disease resistance (Kumar et al., 2019). Fish gut bacteria play a crucial role in nutrient digestion, immunity, and disease prevention in fish (Rawls et al., 2004). However, the impact of SeNPs on fish gut bacteria remains largely unexplored. The current literature suggests that SeNPs supplementation can alter the gut microbiota composition and diversity in fish, although the specific effects may vary depending on the species, dose, and duration of supplementation. Studies are needed to elucidate the underlying mechanisms and potential implications of SeNPs on fish gut bacteria. A few studies have investigated the effect of nanoselenium on fish gut bacteria, which play an important role in nutrient absorption, immunity, and disease prevention in fish.

Yin et al. (2018) investigated the effect of nanoscale selenium on the gut microbiota (Danio rerio). The study found that exposure to nanoscale selenium at concentrations above 50 µg/L resulted in a significant reduction in the diversity and abundance of gut microbiota. In addition, the researchers observed changes in the composition of the gut microbiota, with a decrease in the abundance of beneficial bacteria, such as *Lactobacillus* and an increase in potentially pathogenic bacteria, such as *Pseudomonas*. The study suggests that exposure to nanoscale selenium could have detrimental effects on the gut microbiota of fish, which could impact their overall health and well-being. Several studies have shown that the administration of nanoselenium in fish diets can alter the gut microbiota composition. For example, a recent study conducted by Zhang et al. (2021) investigated the effect of dietary supplementation of SeNPs on the gut microbiota of Nile tilapia. The study

reported a significant change in the gut microbiota composition, with an increase in beneficial bacteria such as *Lactobacillus* and *Bifidobacterium*. Similarly, another study conducted by Zhou et al. (2019) investigated the effect of nanoselenium on the gut microbiota of rainbow trout. The study found that the dietary supplementation of nanoselenium altered the gut microbiota composition, with a decrease in pathogenic bacteria such as *Aeromonas* and *Vibrio*. More research is needed to understand the mechanism behind the changes in gut microbiota composition due to nanoselenium supplementation and its impact on fish health.

The effect of nanoselenium on fish gut bacteria varies depending on the fish species and the duration and dose of nanoselenium supplementation. Some studies suggest that nanoselenium can promote the growth of beneficial bacteria and inhibit the growth of potentially harmful bacteria, while others found no significant effect on the gut microbiota composition. Further research is needed to fully understand the mechanism of action of nanoselenium on fish gut bacteria and its potential applications in aquaculture. The use of nanoselenium has shown promising results in improving the health and growth of fish, however, its impact on the gut microbiota of fish remains largely unexplored. This review aims to summarize the current state of knowledge regarding the effect of nanoselenium on fish gut bacteria. These studies suggest that nanoselenium can have a positive effect on the gut microbiota of fish, leading to improved growth and immune response. Recent studies have shown that selenium supplementation can alter the gut microbiota in animals, including mice and chickens (Yang et al., 2020; Yang et al., 2021). It is possible that Nano-Se supplementation could also impact the composition and function of fish gut microbiota. Therefore, further research is needed to investigate the effects of Nano-Se on fish gut microbiota and the potential implications for fish health and aquaculture practices. Nanoselenium has been found to have potential antimicrobial activity and has been investigated for its effect on fish gut bacteria. Several studies have reported on the impact of nanoselenium on fish gut microbiota.

Zhu et al. (2019), the effects of nanoselenium on the intestinal microbiota of grass carp were investigated. The results showed that nanoselenium had a significant impact on the diversity and composition of the intestinal microbiota. Specifically, the abundance of Proteobacteria and Firmicutes increased while the abundance of Bacteroidetes decreased in response to nanoselenium treatment. Nanoselenium has been found to have varying effects on fish gut microbiota. While some studies have reported positive effects on the abundance of beneficial bacteria, other studies have reported no significant effects. Fish gut bacteria play a critical role in maintaining fish health and promoting growth. However, various factors such as pollution and disease can disrupt the gut microbiota balance, leading to adverse effects on fish health. Nanoselenium has emerged as a potential dietary supplement for fish due to its antioxidant and immune-enhancing properties. In addition to biomedical applications, selenium nanoparticles have also been studied for their potential use in environmental remediation. In a study by Wang et al. (2019), selenium nanoparticles were synthesized and used for the remediation of heavy metal-contaminated soil. The study used a concentration of 2 g/kg of soil of selenium nanoparticles and found that the nanoparticles significantly reduced the bioavailability and toxicity of heavy metals in the soil.

The amount of selenium nanoparticles used in studies varies depending on the application and research objectives. However, it is important to note that the dosage and toxicity of selenium nanoparticles need to be carefully evaluated to ensure their safety and efficacy in various applications. Recent research works have conveyed that the use of nano-selenium as food additive could have positive effects on the nutrition of aquatic animals. In this study, the intestinal bacterial communities associated with low and high concentrations of nano-selenium were characterized, through 16S rRNA V3-V4 sequencing. Selenium nanoparticles (SeNPs) varies based on the specific study and its objectives. SeNPs have potential applications in various fields, including medicine, agriculture and industry, due to their unique properties, such as their antioxidant and antimicrobial activities.

It can be synthesized using various methods, including chemical reduction, microbial synthesis and green synthesis using plant extracts. The size, shape and surface coating of SeNPs can affect their properties, such as their stability, toxicity and bioavailability. SeNPs have shown promising results in vitro and in vivo for various application, such as anticancer agents, antimicrobial agents and antioxidants. The toxicity of SeNPs is a concern and several studies have reported adverse effects on cells and animals at high concentrations or with repeated doses.

In conclusion, SeNPs have potential benefits and challenges for their applications and their synthesis and characterization need further exploration.

CHAPTER 3

3. AIM AND OBJECTIVES:

- Nanoparticle synthesis and characterization.
- To evaluate the toxicity of Chitosan-Selenium Nanoparticle on fish gut bacteria.
- Treatment of culture with nanoparticle and analysis of Cytotoxicity, Lipid peroxidation and ROS formation.

CHAPTER 4

4. MATERIALS AND METHODS:

1. SYNTHESIS OF SELENIUM NANOPARTICLE:

SeNPs were prepared by reducing sodium selenite (Na_2SeO_3) - (10^{-3} M) in 100 mL of distilled water. Ascorbic acid (dark glass) was prepared in (10^{-3} M) in 1 mL of 99 mL of water and add 0.5 g of chitosan. Add dropwise while stirring. Combine sodium selenite and chitosan, mix them in stirrer. While stirring add ascorbic acid (50 mL) by titrating method. It changes to brick red colour. Centrifuge it at 8000 rpm for 20 mins, in 27°C .



Figure 4.1 Synthesized CS-SeNPs

2. CHARACTERIZATION OF CS-SeNPs:

- Characterization is done by using UV-Spectroscopy (UV-1800) Shimadzu Japan which is a quantitative technique used to measure how much a chemical substance absorbs light.

- Particle Size Analysis (PSA) nanoparticle PSA HORIBA, Japan which determines the size range and the average or mean size of the particles in a powder or liquid sample.
- Fourier - transform infrared spectroscopy (FTIR) Agilent Cary 630- ATR is done to identify compounds.
- Transmission electron microscope JeOL Japan is done to characterize the morphological shape and size of the materials.

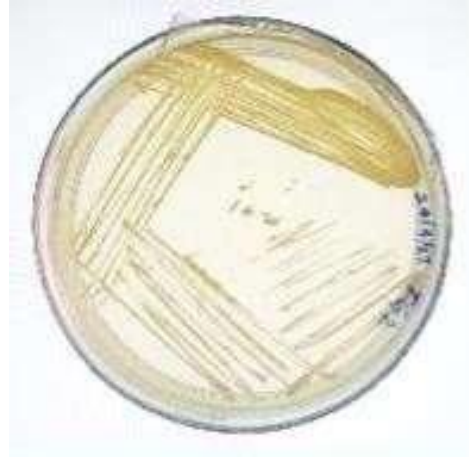
4.3 EXPOSURE OF CS-SeNPs TO FISH GUT BACTERIA:

Fish gut isolates are being maintained in NB with 50 % of seawater. Exposure to selenium nanoparticles can potentially affect the abundance and diversity of gut bacteria, which can in turn affect the overall health of the fish.

FG1



FG2



FG3



FG6



Figure 4.2 Fish gut isolates (FG1, FG2, FG3 & FG6)

4. TOXICITY ANALYSIS:

1. CYTOTOXICITY:

Cytotoxicity refers to the ability of a substance or agent to cause damage or death to cells. To determine the toxicity effect of CS-SeNPs, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) dye reduction assay was performed. Reduction of MTT to purple coloured formazan by mitochondrial dehydrogenase, an enzyme presents in the mitochondria of viable cells denote cell viability. Add 100 μL culture and 50 μL NP + 50 μL Tx after adding, incubate and take reading in the ELISA reader, after 24 hrs add MTT of 20 μL and kept in dark incubation, after 4 hrs take readings in the ELISA reader. Results were calculated.

2. LIPID PEROXIDATION:

Lipid peroxidation is a process that occurs when free radicals react with the polyunsaturated fatty acids (PUFAs) present in cell membranes. It is a chain reaction that leads to the destruction of the membrane lipids, and it can result in cellular damage or death. The process of lipid peroxidation begins when free radicals, such as hydroxyl radicals ($\text{OH}\cdot$) or peroxy radicals ($\text{ROO}\cdot$), attack the double bonds in the PUFAs. This leads to the formation of lipid radicals ($\text{L}\cdot$) which are highly reactive and can further propagate the chain reaction. As the chain reaction continues, it leads to the production of lipid peroxides (LOOH) which are highly toxic and can cause damage to cellular structures and functions. Bacterial culture 200 μL + CS-SeNPs and Tx 100 μL were added in 96 well plate for 4-6 hrs and then 500 μL of 0.1 M HCL is added. It is incubated for 20 mins at room temperature. After incubation, 900 μL of 0.025 M. TBA was added. This is incubated 65 mins at 37 $^{\circ}\text{C}$. 400 μL of 10 mM. PBS were added and the readings are recorded at 520 or 540 nm.

4.4.3 ROS ESTIMATION:

ROS (Reactive Oxygen Species) estimation is the process of measuring the levels of highly reactive molecules in biological systems that contain oxygen. Production of ROS was measured spectrophotometrically using the fluorescent probe, 2,7- dichlorofluorescein diacetate (DCFH-DA). DCFH-DA can diffuse readily through the cell membrane and is hydrolysed by cellular esterase to form non-fluorescent compound 2',7'-dichlorofluorescein (Wang and Joseph, 1999). 5 mL of CS-SeNPs is taken and treated with bacterial cells and it is incubated in with 5 μ L of DCFH-DA (100 μ M) for 30 mins. Fluorescence was measured using synthesis TM H1 Hybrid multimode reader (530 nm and 485 nm).

CHAPTER 5

5. RESULTS AND DISCUSSION:

1. CHARACTERIZATION OF SYNTHESIZED CS-SeNPs

1. UV VISIBLE SPECTROSCOPY:

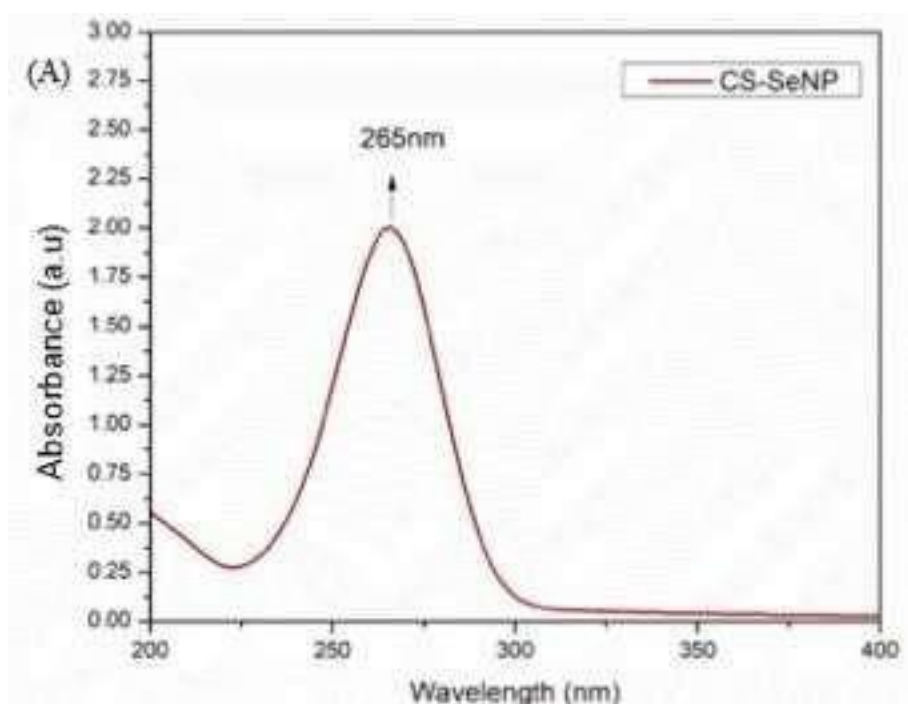


Figure 5.1 UV absorption spectrum of CS-SeNPs

The UV visible spectrum (Fig 5.1) of synthesized nanomaterial revealed absorption peaks at 265 nm which is confirmatory for the presence of selenium nanoparticles. It is based on the absorption of ultraviolet light or visible light that results in the production of spectrum. It comprises electromagnetic radiation of relatively high energy with the wavelength ranges of 200-400 nm called ultraviolet spectroscopy and 400-800 nm called visible spectroscopy.

5.1.2 FTIR:

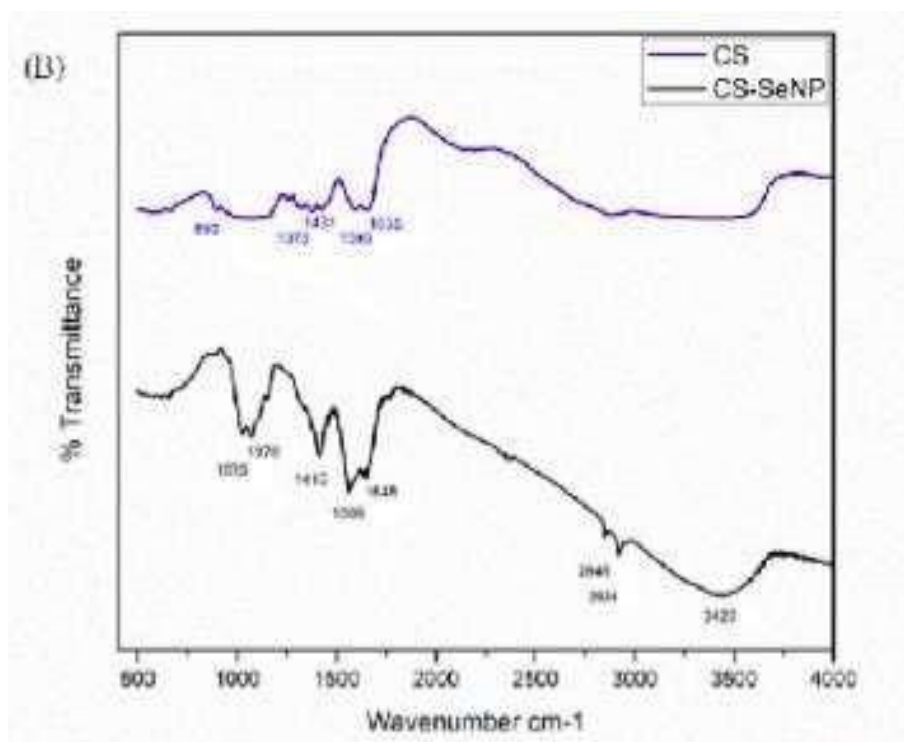


Figure 5.2 FTIR Spectrum of Chitosan and Synthesised CS-SeNPs

In the FTIR Spectrum (Fig 5.2), peaks at 1648 cm^{-1} and 1655 cm^{-1} correspond to asymmetrical and symmetric stretching vibrations of COO^- . The peak obtained at 1378 cm^{-1} indicates the symmetric stretch of COO^- in Chitosan. The shift in peak to 1415 cm^{-1} indicated the involvement of COO^- groups in the synthesis of CS-SeNF. The band at and 3420 cm^{-1} corresponds to the O-H stretching. The IR band obtained at 2849 cm^{-1} , is indicative of the presence of Aliphatic C-H vibrations. The band at 890 cm^{-1} correspond to $=\text{C-H}$ out of plane bending and $-(\text{CH}_2)_n$. FTIR is to identify chemical bonds by producing an infrared absorption spectrum. It is mainly characterized using functional groups.

5.1.3 PARTICLE SIZE ANALYSIS:

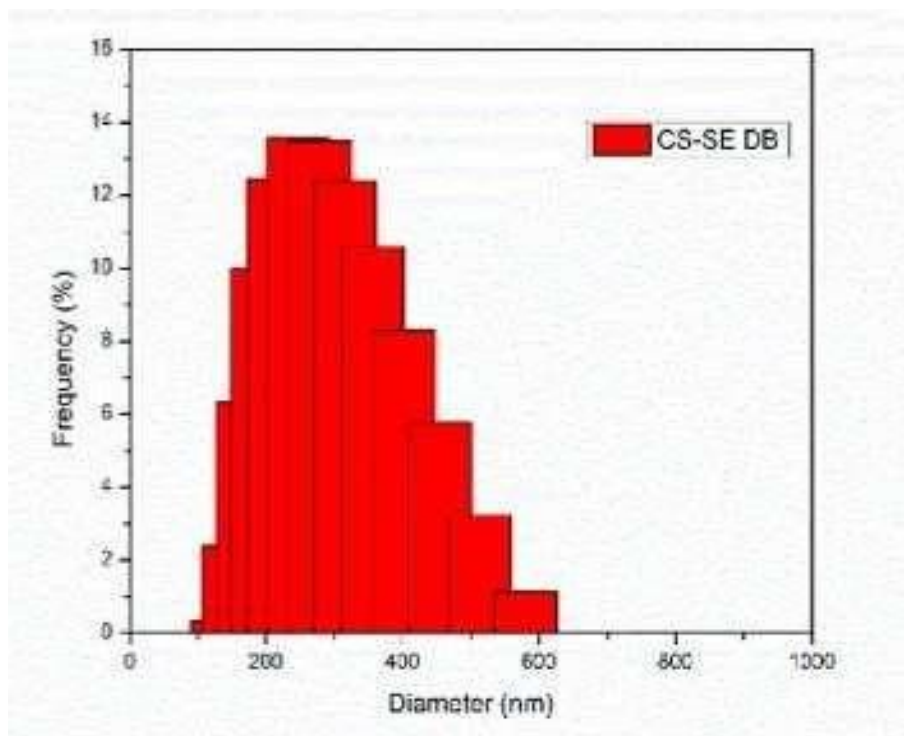


Figure 5.3 Particle Size measurements.

The CS-SeNPs synthesized using dark incubation method with 6 h incubation time was found to have a mean Particle size (Fig 5.3) of 186.3 nm. When a beam of light is scattered by a group of particles, the angle of light scattering is inversely proportional to particle size.

5.1.4 ZETA POTENTIAL OF CS-SeNPs:

The CS-SeNPs synthesized using dark incubation method with 6 h incubation time was found to have a mean a Zeta potential (Fig 5.4) value of 49.1 mV. Zeta potential or electro kinetic potential between shear plane and electro-neutral region of motion of the solution. It ranges from +100 mV to - 100 mV.

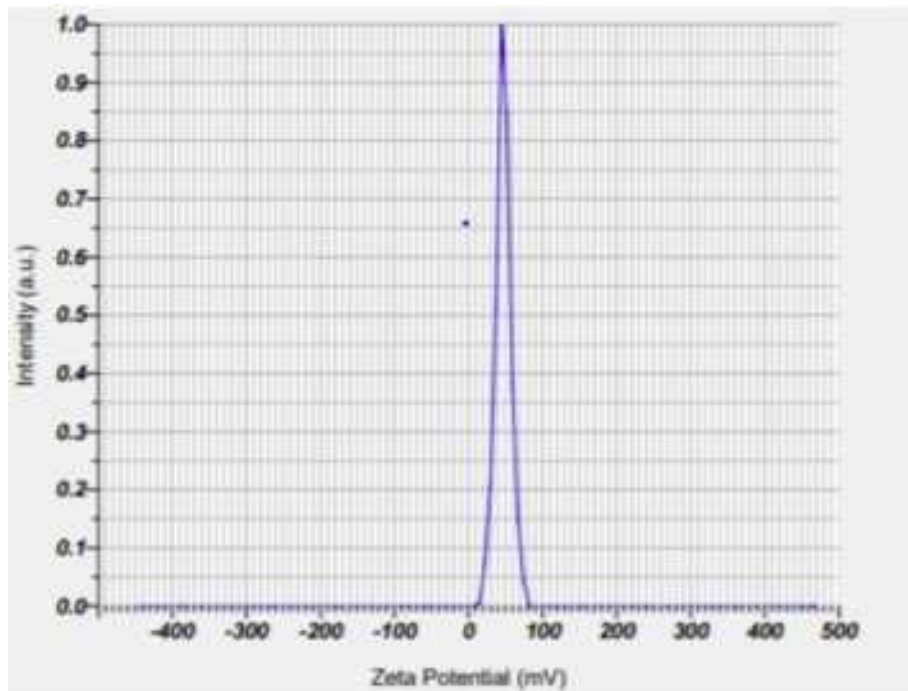
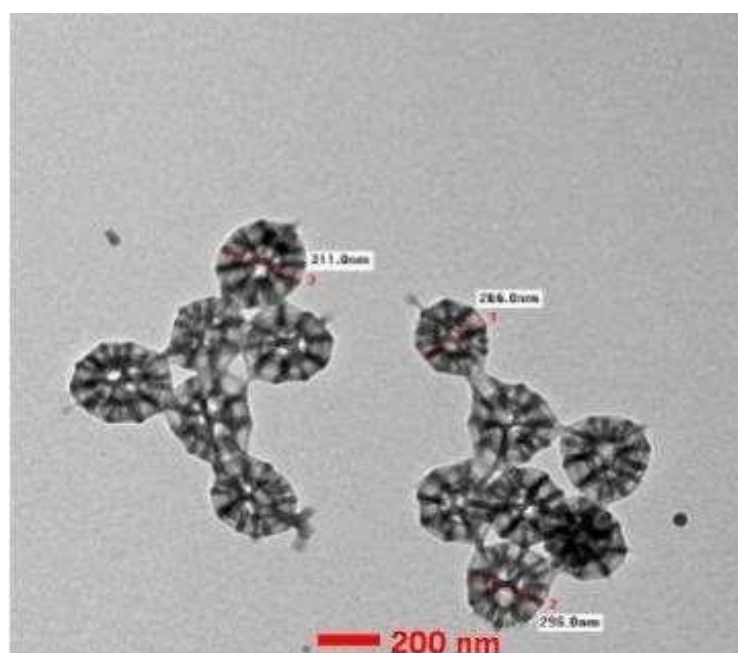


Figure 5.4 Zeta Potential of CS-SeNPs synthesized by dark incubation method.

5.1.5 TEM:

The principle of TEM is based on the interaction of a beam of high-energy electrons with a thin sample, which is typically less than 200 nm in thickness. As the electrons pass through the sample, they interact with the atoms and scatter in different directions, creating a complex pattern that can be detected by a series of lenses and sensors. TEM Images (Fig 5.5) show Polydisperse Nanoflowers in the size range 260-311 nm.

(A)



(B)

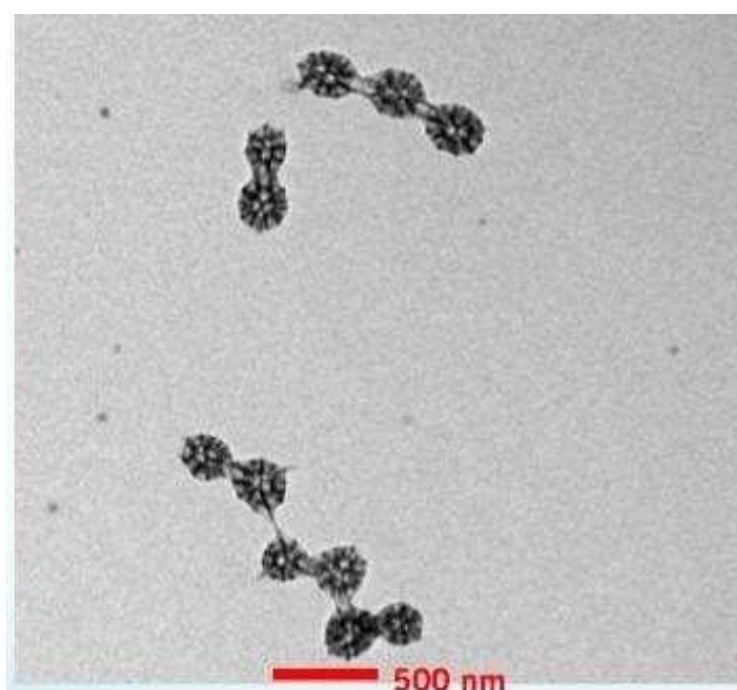


Figure 5.5 Transmission Electron micrographs of CS-SeNPs synthesized by dark incubation (6 h).

2. CYTOTOXICITY TESTING:

1. CYTOTOXICITY ASSAY

TABLE: 5.1

CONCENTRATION $\mu\text{g/mL}$	FG1	FG2	FG3	FG6
0	0	0	0	0
15.625	15.92	14.22	11.5	9.66
31.25	19.52	19.20	32.8	15.87
62.5	19.54	22.30	48.1	35
125	21.14	24.46	53.6	58.9
250	35.24	30.96	70.2	63.8
500	63.20	70.89	87.6	82.6
1000	71.20	78.29	92.8	93.6

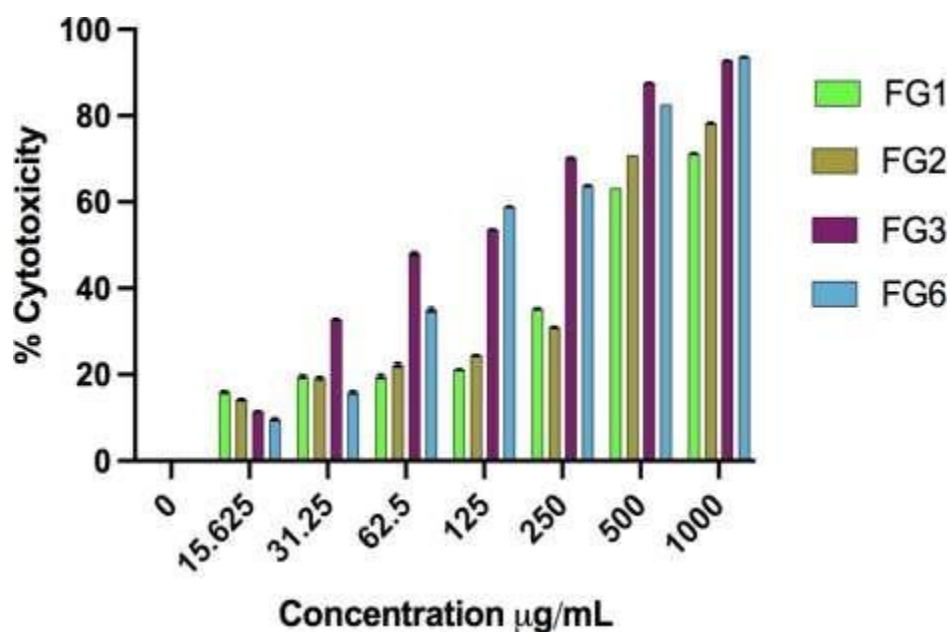


Figure 5.6 Cytotoxicity analysis of CS-SeNPs against FG1, FG2, FG3, FG6.

At the minimum concentration of 15.625 $\mu\text{g/mL}$, the CS-SeNPs showed a cytotoxicity % of FG1 - 15.92 %, FG2 - 14.22 %, FG3 - 11.5 %, FG6 - 9.66 % and at the maximum concentration of 1000 $\mu\text{g/mL}$, the CS-SeNPs showed a cytotoxicity % of FG1 - 71.20 %, FG2 - 78.29 %, FG3 - 92.8 %, and FG6 - 93.6 %.

FG6 - 93.6 % which collides with the trend of the positive control Triton-X. This concludes, the cytotoxicity is evidentially increasing with respect to the increase in the CS-SeNPs concentration.

5.2.2 LIPID PEROXIDATION ASSAY:

TABLE: 5.2

FG1

CONCENTRATION µg/mL	Triton-X	CS-SeNPs
0	0	0
15.625	8.96	18.65
31.25	20.62	23.98
62.5	32	25.87
125	43	37.86
250	51.08	49.22
500	69	60.56
1000	74.23	72.97

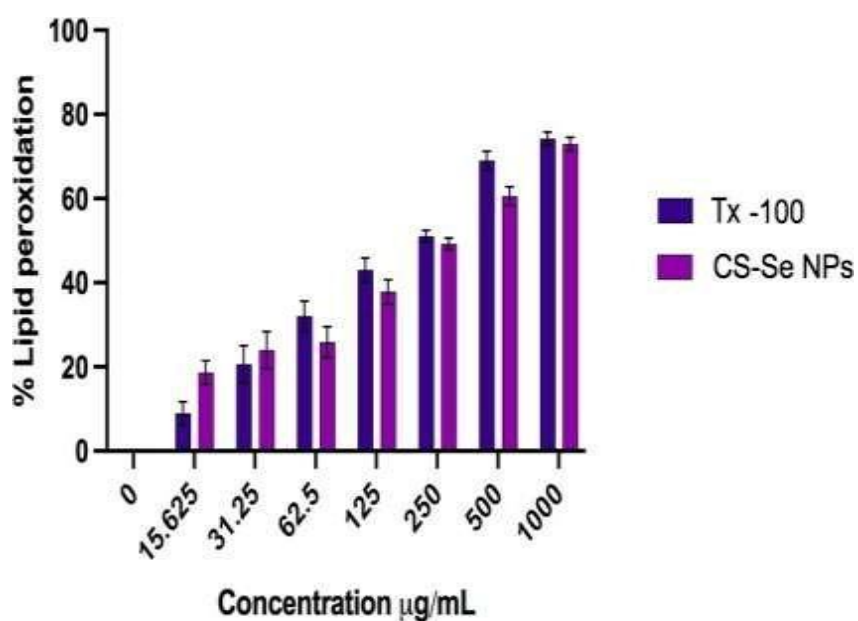


TABLE: 5.3

FG2

CONCENTRATION µg/mL	Triton-X	CS-SeNPs
0	0	0
15.625	17.53	4.78
31.25	22	12.78
62.5	40	22.56
125	49.97	27.89
250	61	50.89
500	72	63.90
1000	90.23	72.90

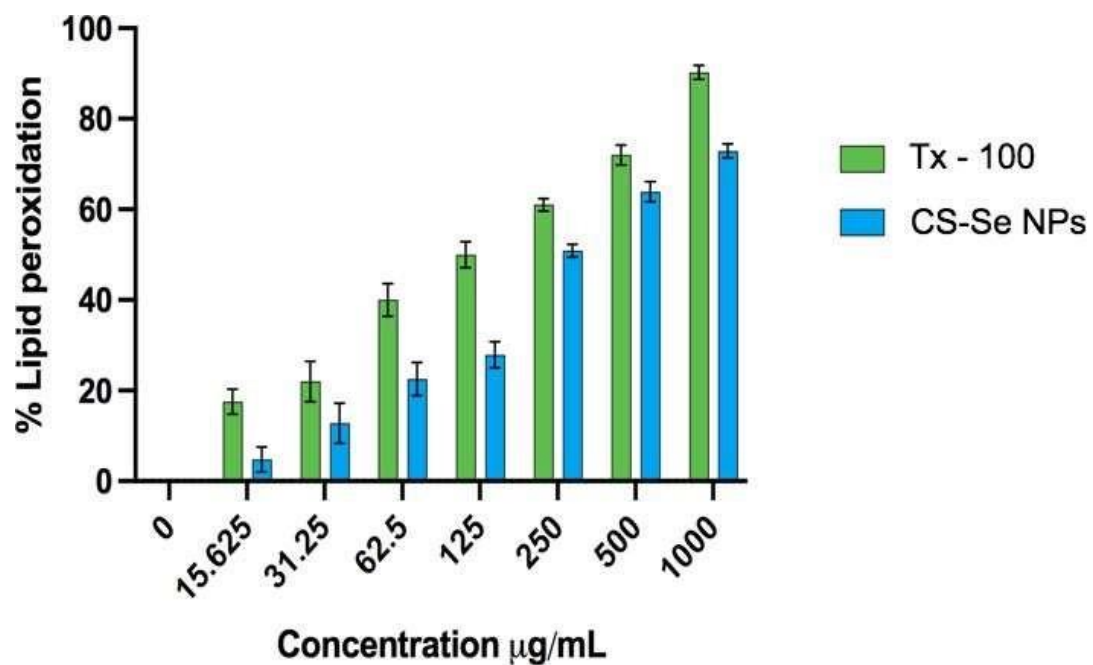


TABLE: 5.4

FG3

CONCENTRATION μg/mL	Triton-X	CS-SeNPs
0	0	0
15.625	20.89	11.12
31.25	28.98	20.89
62.5	43.98	27.98
125	52.94	46.98
250	69.56	59.08
500	78.89	72.99
1000	92.76	81.99

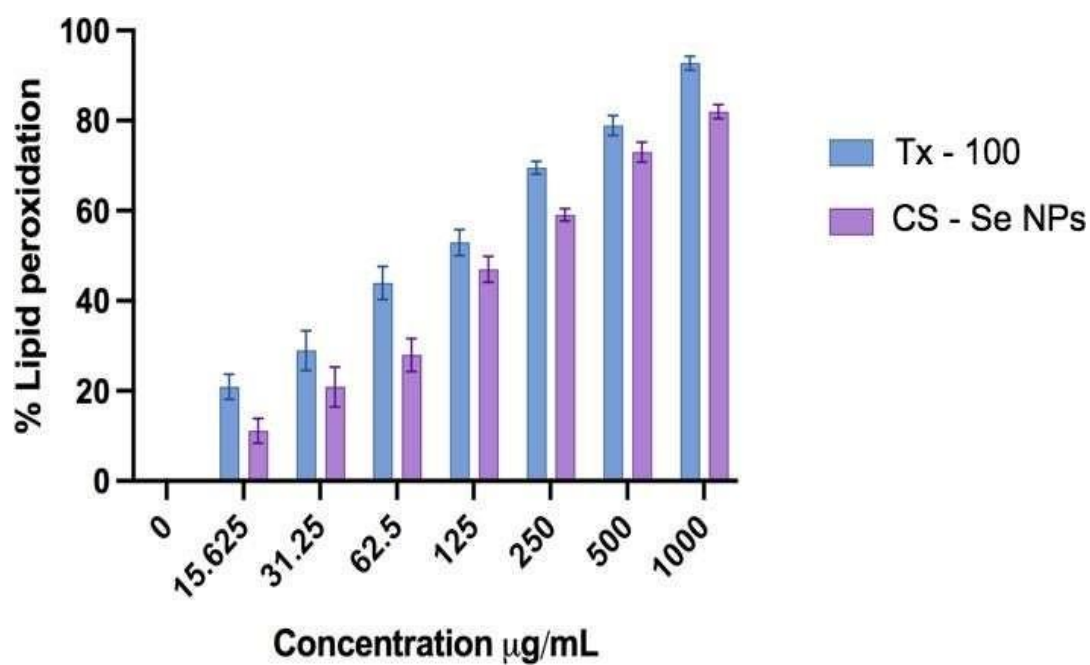


TABLE: 5.5

FG6

CONCENTRATION $\mu\text{g/mL}$	Triton-X	CS-SeNPs
0	0	0
15.625	16.55	2.20
31.25	22	18.90
62.5	43.90	32.79
125	52.80	41.98
250	74.20	53.20
500	88.70	69.76
1000	93.80	76.98

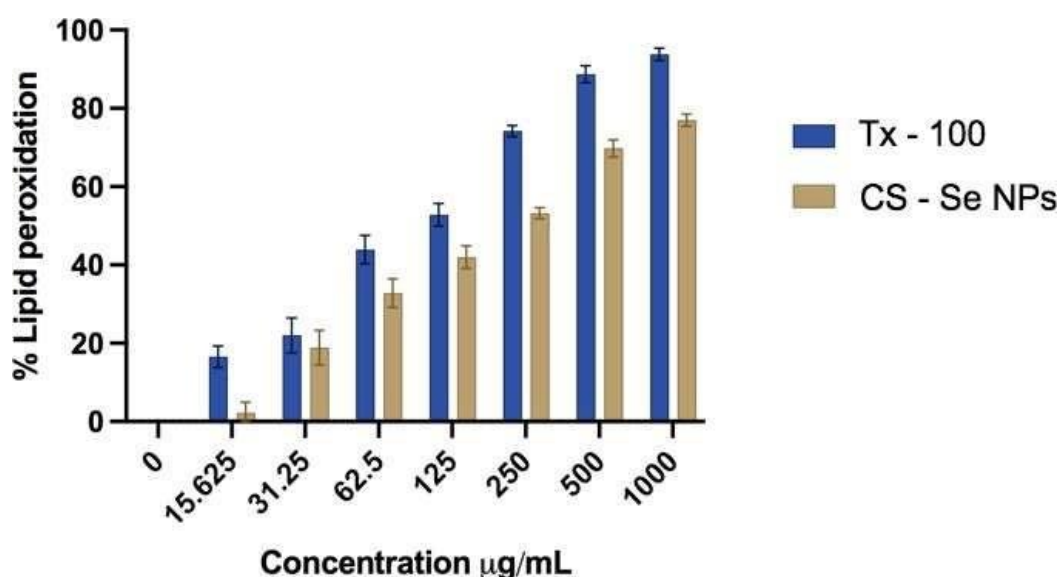


Figure 5.7 Lipid peroxidation analysis of CS-SeNPs against FG1, FG2, FG3, FG6.

At the minimum concentration of $15.625 \mu\text{g/mL}$, the CS-SeNPs showed a cytotoxicity % of FG1 - 18.65 %, FG2 - 4.78 %, FG3 - 11.12 %, FG6 - 2.20 % and at the maximum concentration of $1000 \mu\text{g/mL}$, the CS-SeNPs showed a cytotoxicity % of FG1 - 72.97 %, FG2 - 72.90 %, FG3 - 81.99 %, FG6 - 76.98 % which collides with the trend of the positive control Triton-X. This

concludes, the lipid peroxidation is evidentially increasing with respect to the increase in the CS-SeNPs concentration.

5.2.3 ROS ESTIMATION:

TABLE: 5.6

FG1

CONCENTRATION $\mu\text{g/mL}$	Triton-X	CS-SeNPs
0	0	0
15.625	16.55	5.9
31.25	22	11.3
62.5	43.9	33.79
125	52.8	41.78
250	74.2	53.2
500	88.7	60.26
1000	93.8	76.29

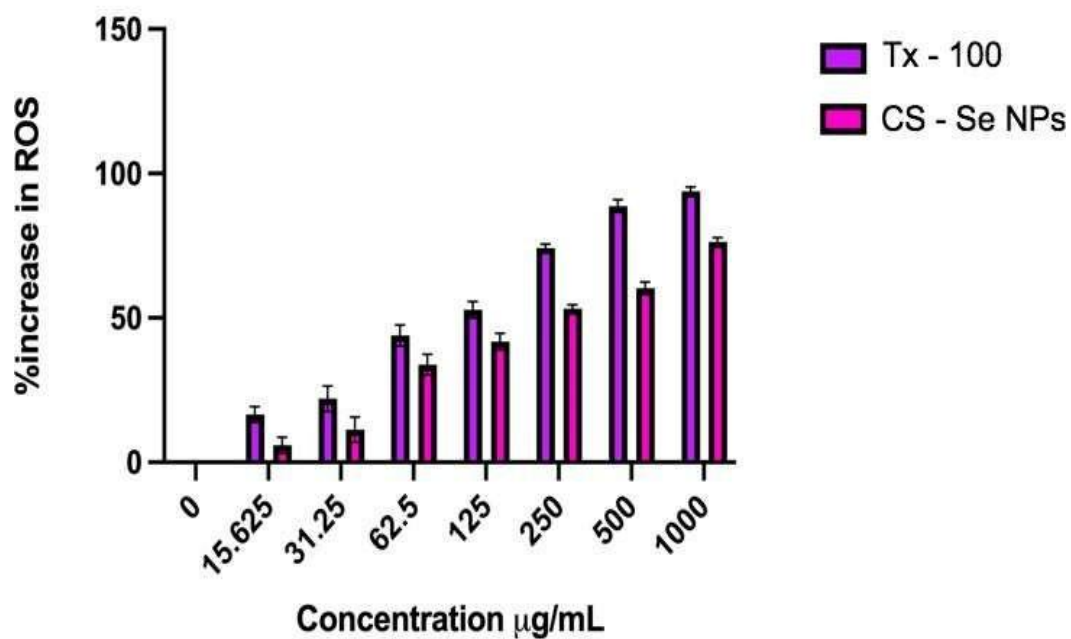


TABLE: 5.7

FG2

CONCENTRATION $\mu\text{g/mL}$	Triton-X	CS-SeNPs
0	0	0
15.625	25	8.9
31.25	28.9	12.3
62.5	39.59	26.20
125	69.45	44.42
250	73.50	65.6
500	90	81.9
1000	97	90.4

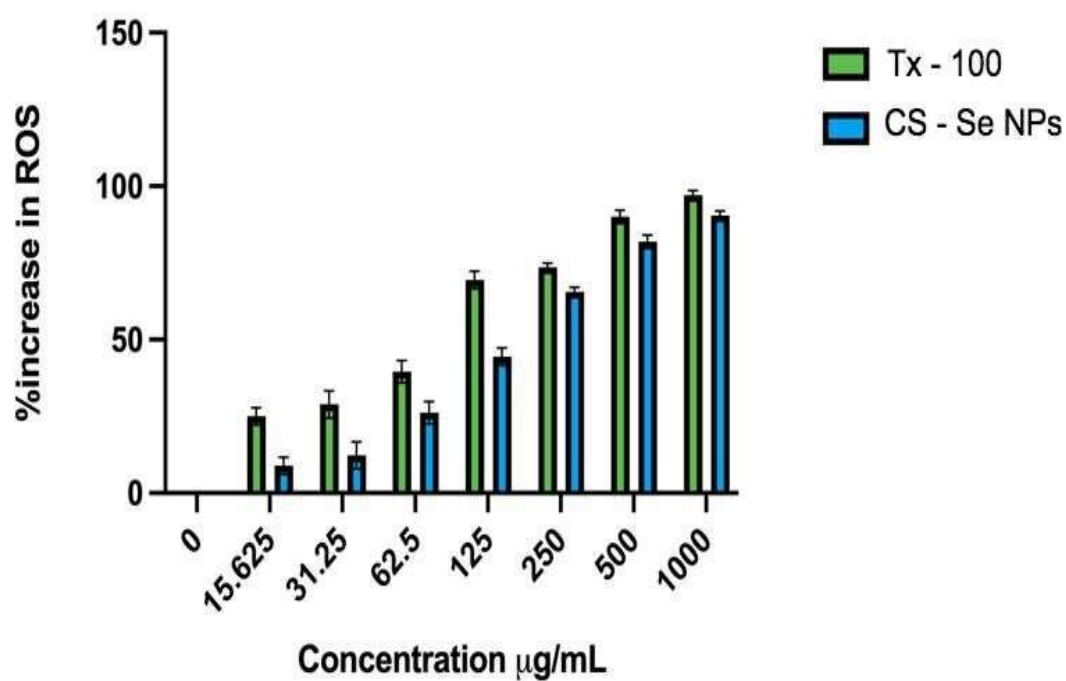


TABLE: 5.8

FG3

CONCENTRATION $\mu\text{g/mL}$	Triton-X	CS-SeNPs
0	0	0
15.625	30.2	8.20
31.25	37.9	22.5
62.5	43.7	25.8
125	72.8	50.8
250	88.5	63.92
500	93.8	87.9
1000	97.27	93.90

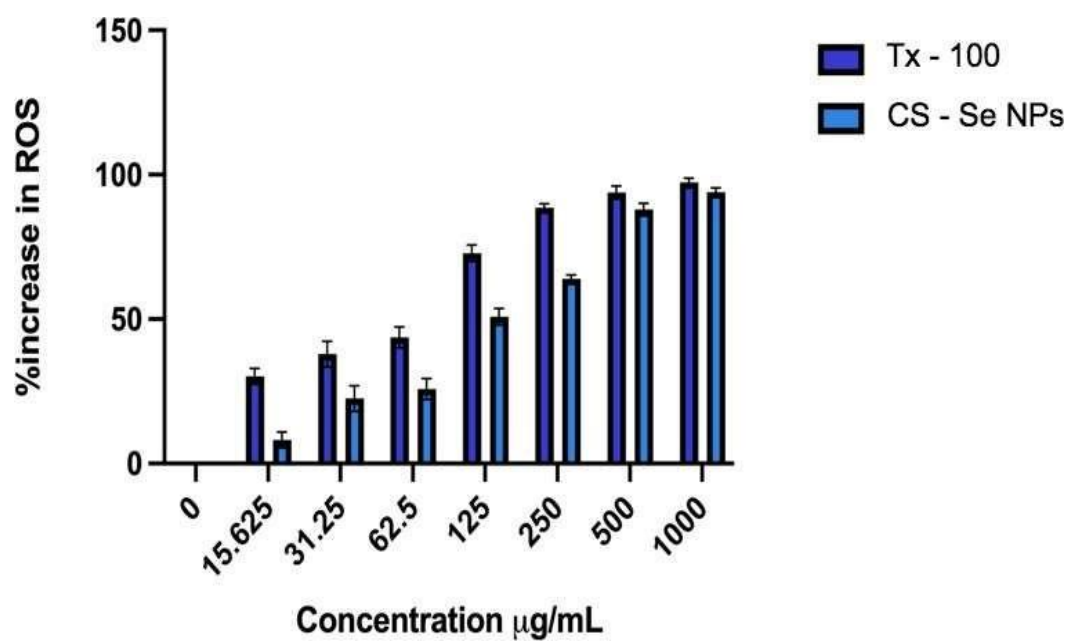


TABLE :5.9

FG6

CONCENTRATION $\mu\text{g/mL}$	Triton-X	CS-SeNPs
0	0	0
15.625	33	5.7
31.25	37.98	44.80
62.5	43.22	52.9
125	83.90	64
250	87.85	84.78
500	94	92.97
1000	97.9	95.99

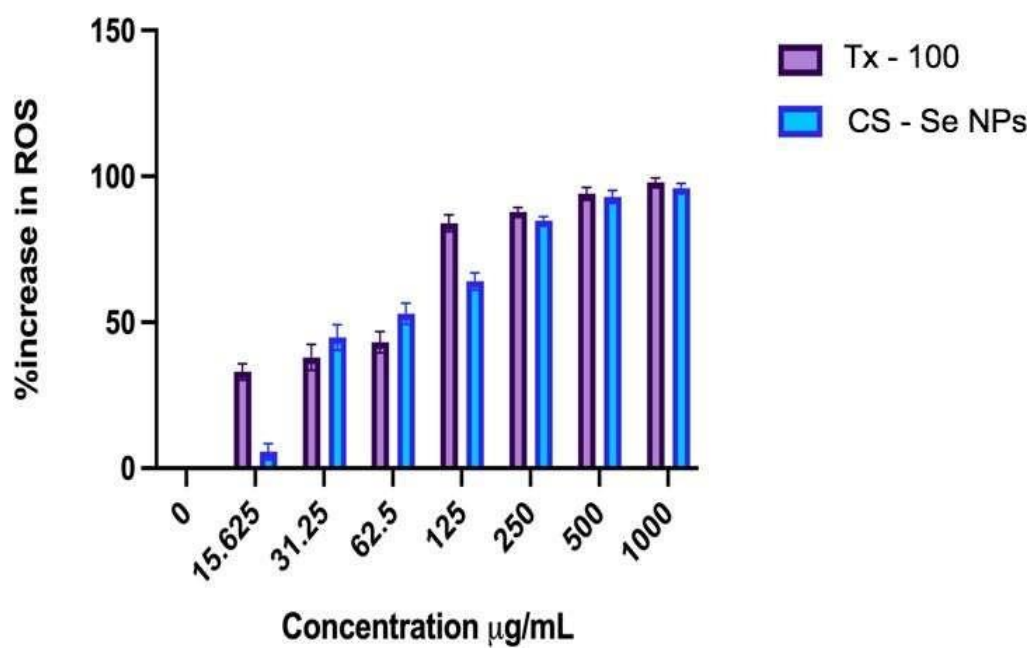


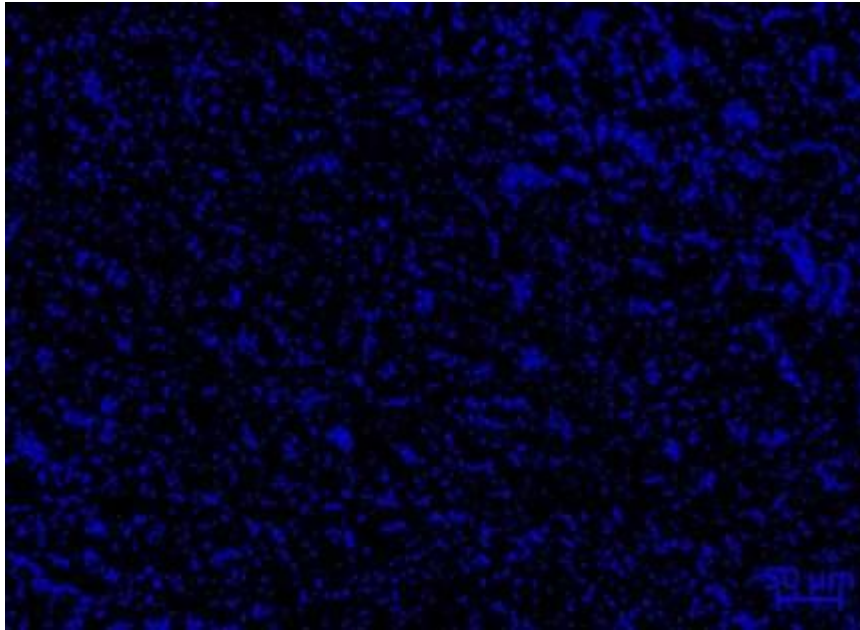
Figure 5.8 ROS Estimation analysis of CS-SeNPs against FG1, FG2, FG3, FG6

At the minimum concentration of 15.625 $\mu\text{g/mL}$, the CS-SeNPs showed a cytotoxicity % of FG1- 5.9 %, FG2- 8.9 %, FG3- 8.20 %, FG6- 5.7 % and at the maximum concentration of 1000 $\mu\text{g/mL}$, the CS-SeNPs showed a cytotoxicity % of FG1- 76.29 %, FG2- 90.4 %, FG3- 93.90 %, FG6- 95.99 % which collides with the trend of the positive control Triton-X. This concludes, the ROS estimation is evidentially increasing with respect to the increase in the CS-SeNPs concentration.

5.3 DAPI STAINING:

DAPI staining is a commonly used technique in microscopic to visualize DNA within cells and tissues. DAPI (4,6-diamidino-2-phenylindole) is a blue-fluorescent DNA stain that exhibits enhancement of fluorescence upon binding to AT regions of dsDNA. It is used to visually measure degree of cellular apoptosis. At the same time, for normal cells, round nucleus is stained uniformly and its margin is clear. But, for apoptotic cells, the margin of nucleus is abnormal and disrupted. The degree of fluorescence indicates the degree of apoptosis. Apoptotic cells produce high blue fluorescence as the apoptotic cell membrane is compromised, more DAPI enters the cell and stains a stronger blue color.

(A)



(B)

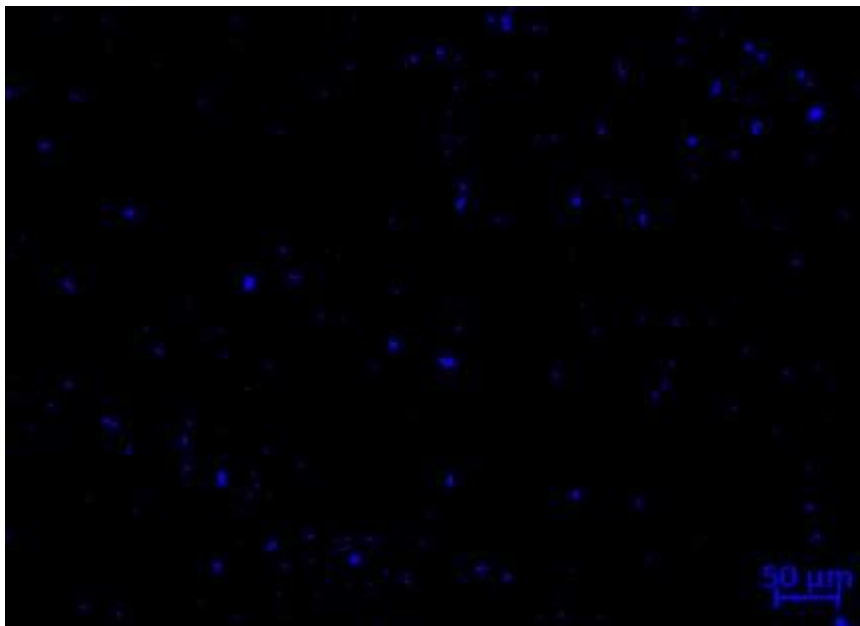


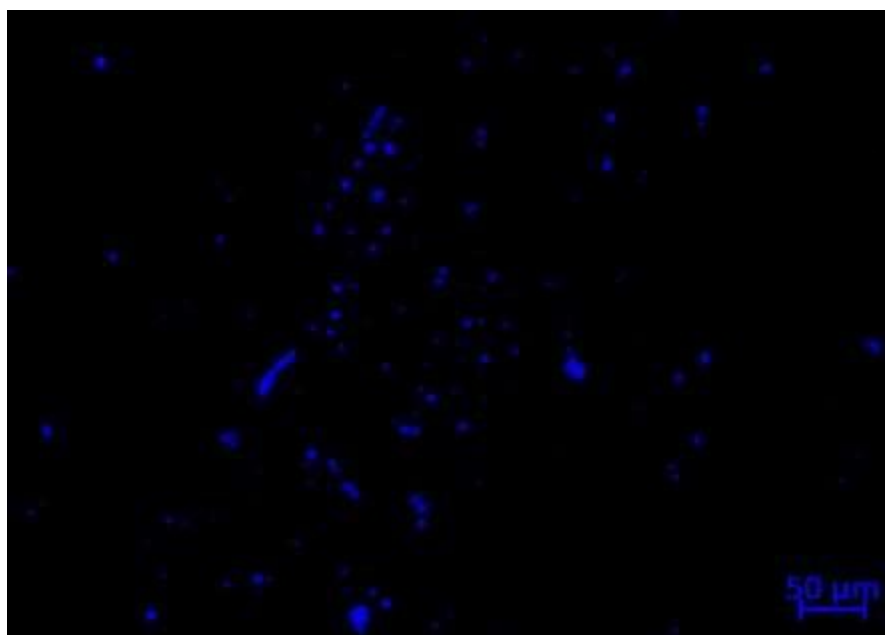
Figure 5.9 shows FG1 BACTERIA

(A)

CONTROL (UNTREATED)

(B) *TREATED WITH IC₅₀: 500 μg/mL OF CS-SeNPs*

(A)



(B)

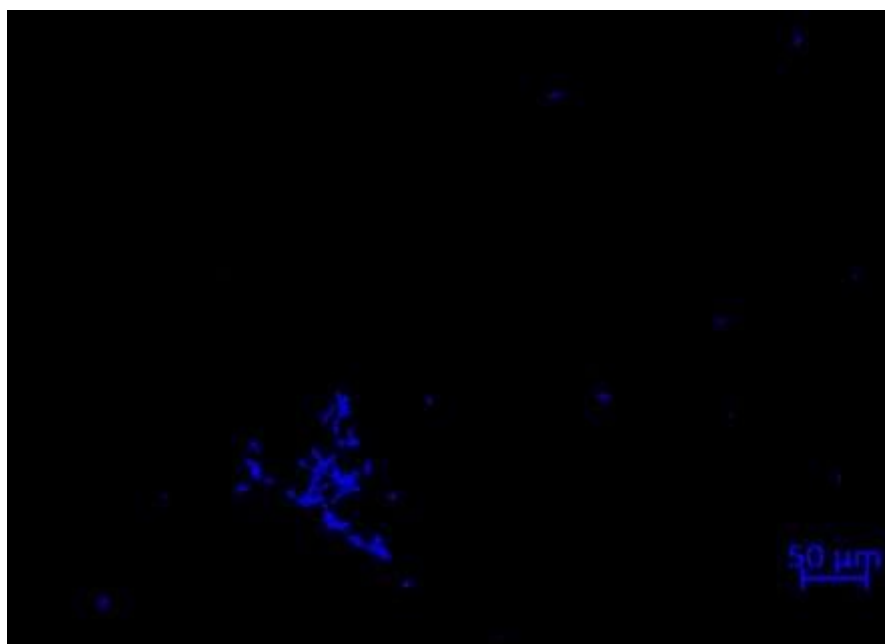


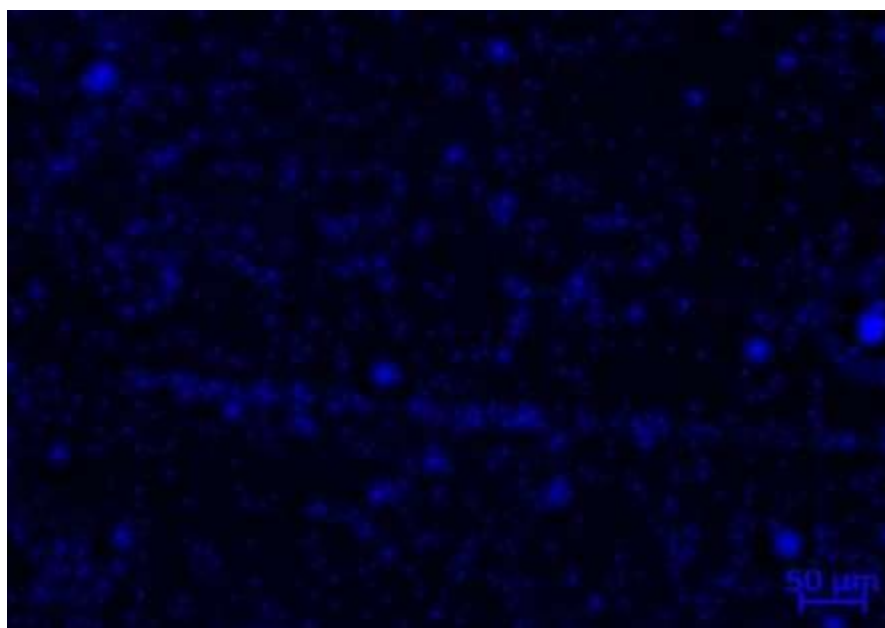
Figure 5.10 shows FG2 BACTERIA

(A)

CONTROL (UNTREATED)

(B) *TREATED WITH IC₅₀: 500 μg/mL OF CS-SeNPs*

(A)



(B)

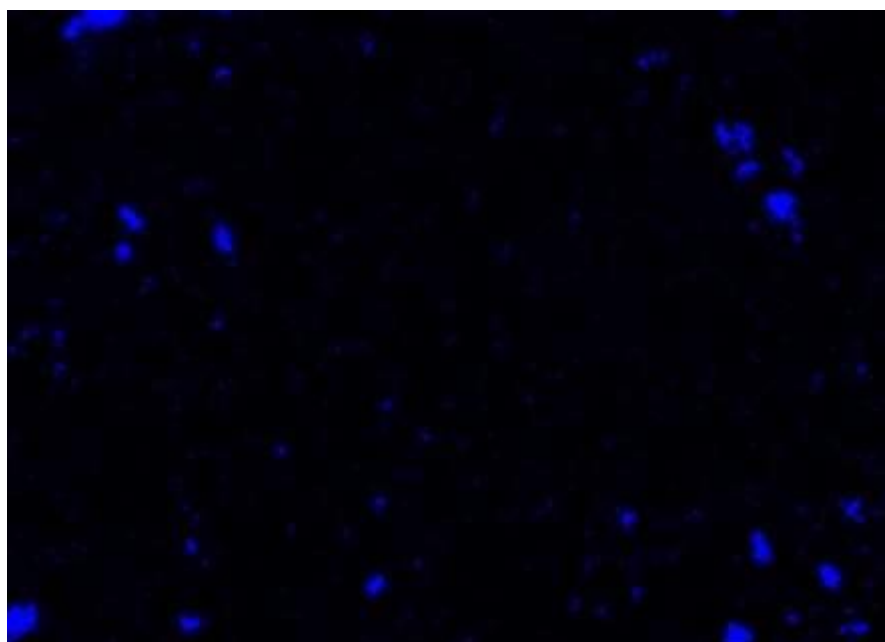


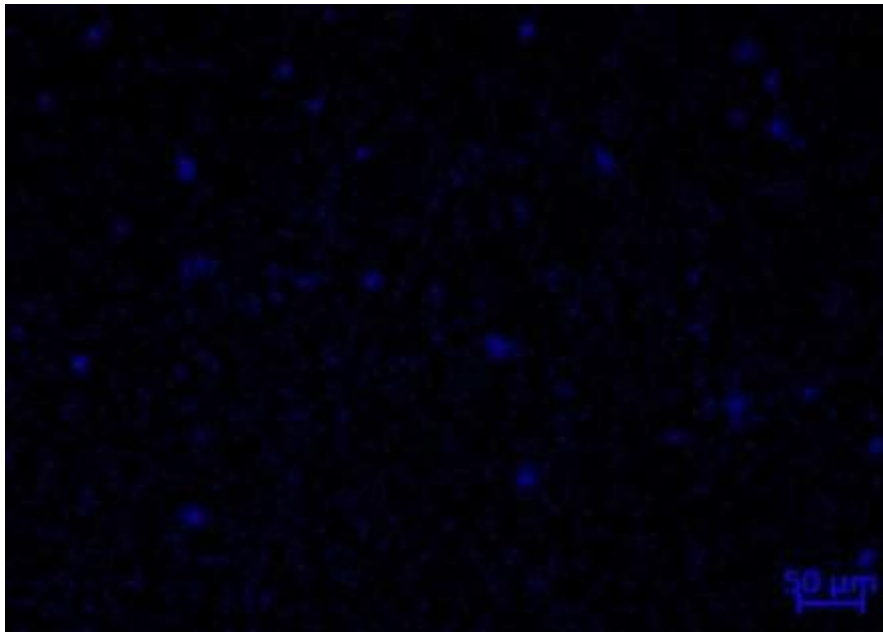
Figure 5.11 shows FG3 BACTERIA

(A)

CONTROL (UNTREATED)

(B) *TREATED WITH IC₅₀: 62.5 μg/mL OF CS-SeNPs*

(A)



(B)

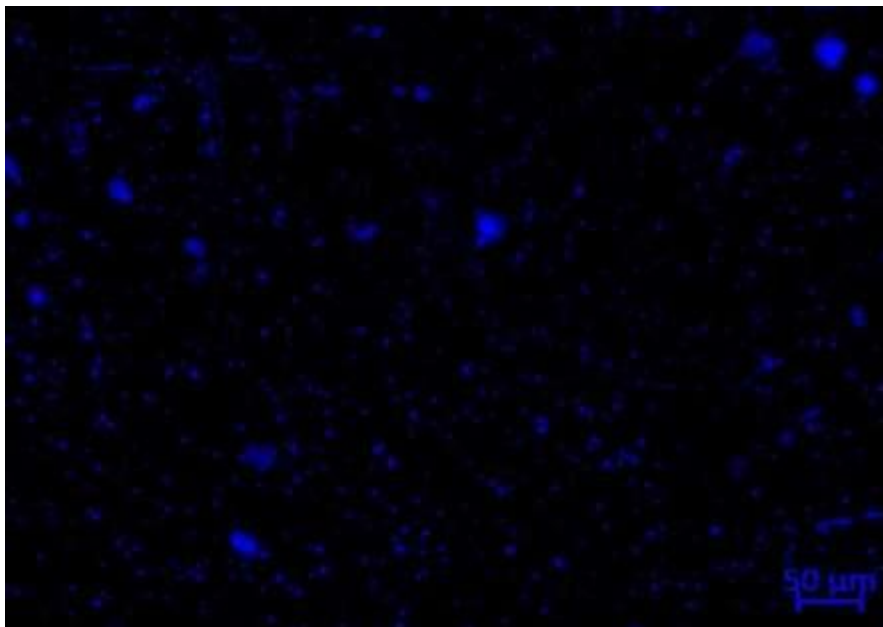


Figure 5.12 shows FG6 BACTERIA

(A)

CONTROL (UNTREATED)

(B) *TREATED WITH IC₅₀: 125 μg/mL OF CS-SeNPs*

CHAPTER 6

6. SUMMARY AND CONCLUSION

CS-SeNPs were prepared and characterized using various spectroscopic techniques. The Exposure of CS-SeNPs to that the toxicity is dependent on concentration and duration. CS-SeNPs were found to have a considerable effect on the levels of ROS. Flow cytometric analysis indicated the level of damage upon exposure to CS-SeNPs. The results indicate that interaction with CS-SeNPs results in distortion of morphological characteristics after 96 h. The changes observed in cell viability, activity of MTT, ROS and MDA establish clear endpoint on CS-SeNPs toxicity.

CHAPTER 7

7. REFERENCES

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