Journal of Pharmaceutical Research International

32(27): 33-39, 2020; Article no.JPRI.59701
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Assessment of Toxicity of Selenium Nanoparticle Varnish Using HepG2 Cell Lines: *In vitro* Study

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Authors' contributions

This work was carried out in collaboration among all authors. Author MI conducted literature search, wrote the protocol and the first draft of the manuscript. Author SSR designed the study, performed the statistical analysis and critically revised the paper. Authors IMA and RPK critically revised the paper. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2020/v32i2730853
Editor(s):
(1) Dr. Vasudevan Mani, Qassim University, Kingdom of Saudi Arabia.
Reviewers:
(1) Sandra Machado Lira, Centro Universitário Maurício de Nassau, Brasil.
(2) Mohammadreza Mohammadabadi, Shahid Bahonar University of Kerman, Iran.
Complete Peer review History: http://www.sdiarticle4.com/review-history/59701

Original Research Article

Received 12 June 2020
Accepted 18 August 2020
Published 07 November 2020

ABSTRACT

Selenium, an essential trace element, plays an important role in mammalian biology. Selenium nanoparticles (SeNPs) have gained significant importance because of its bioavailability, least toxicity, its interaction with proteins and its biocompatibility. The objective of the present study is to assess the cytotoxicity of SeNPs by testing on HepG2 cell line. The cytotoxicity of nanoparticles on HepG2 cell line was studied by MTT assay. Cytotoxicity was determined using Graph pad prim5 software. The SeNPs showed cytotoxic activity against HepG2 cell line with 77%, 63% and 33.7% of cell viability at 2µg/ ml, 4µg/ml and 30µg/ml concentration respectively. Biogenic SeNPs exhibited cytotoxic activity against the HepG2 cell line and hence warrants further research regarding its biosafety and potential oral antimicrobial agent.

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Keywords: Cell line; nanoparticles; selenium; toxicity.

1. INTRODUCTION

Nanotechnology is a multidisciplinary scientific area, which employs a diverse array of tools and techniques derived from engineering, physics, chemistry and biology [1–3]. Advancements in nanoscience and nanotechnology have made it possible to manufacture and characterize sub-micron bioactive carriers on a routine basis. The delivery of bioactives to target sites inside the body and their release behavior is directly affected by particle size [4,5]. Compared to micrometer-sized carriers, nanocarriers provide more surface area and have the potential to increase solubility, enhance bioavailability, improve controlled release and enable precision targeting of the entrapped material to a greater extent [2,5].

Selenium (Se) is an essential trace element that is crucial for many cellular functions by the incorporation of selenoproteins [6]. Selenium nanoparticles (SeNPs) are gaining importance because of its least toxicity, bioavailability, its interaction with proteins and biocompatibility when compared to organic and inorganic selenium [7]. They are known for its potent anticancer activity at high dosage [8]. SeNPs own excellent photoelectric and semiconductor properties [9].

Selenium as a dietary supplement has been demonstrated to reduce the risks of various types of cancers including prostate cancer, lung cancer, and esophageal and gastric-cardiac cancers. Selenium-enriched probiotics have been shown to strongly inhibit the growth of pathogenic *Escherichia coli* in vivo and in vitro. In vivo, mice were fed with and without selenium-enriched probiotics for 28 days and then inoculated with *E. coli*; mortality of the treated group was the lowest [10]. Biomedical applications of SeNPs include drug and targeted gene delivery, anticancer activity, antibacterial activities, antiinflammatory activities and biosensors [11]. Historically it was believed that Se toxicity was due to an alteration of the tertiary structure of proteins when Se substituted for S, but a more general mechanism involving oxidative stress or impaired immune function has also been proposed [12].

SeNPs can be synthesised by various methods such as laser ablation method, microwave-assisted method, by chemical reduction, electro-deposition method and solvothermal synthesis. But stringent synthetic conditions, such as harsh chemicals, acidic pH, and high temperature restrict their use in biomedical application [13].

Toxicity of selenium mainly thought to be due to its pro-oxidant ability to catalyze the oxidation of thiols and simultaneous Free Radical Biology & Medicine [14]. The antioxidant and pro-oxidant effects, or bioavailability and toxicity, of selenium depend on its chemical form. Selenomethionine is the predominant chemical form of selenium in foodstuffs and selenium-enriched yeast [15]. Some studies on the toxicity of selenium nanoparticles indicate the greater toxicity of chemically generated selenium nanoparticles while oxyanions of selenium have been found to be more highly toxic to rats as compared to nano-Se. We have successfully completed numerous epidemiological and invitro studies for the betterment of our community [16–33]. This paper aims at the current understanding of the toxicity of biogenic selenium (Se) nanoparticle varnish.

2. MATERIALS AND METHODS

Cell Culture: The cell line HepG2 was purchased from NCCS, Pune, India. The cells were grown in Dulbecco's modified eagle media supplemented with 10% Fetal Bovine Serum (FBS), 1% of Penicillin and Streptomycin and grown at 37°C in a humidified atmosphere of 95% air and 5% CO2. The cells were allowed to grow to 70-80% confluence and were seeded at a density of 1 10 6 cells per well and incubated for 24 h in 95% air and 5% CO 2 incubator. Reagents:

1. MTT (3-[4, 5-dimethylthiazol-2-yl]2, 5-diphenyl tetrazolium bromide):5mg/ml of serum-free DMEM medium.
2. Solubilising solution: Dimethyl sulfoxide
3. Phosphate buffered saline (PBS) (pH 7.4): As described under cell culture reagents

Principle: The assay is based on the most viable cell mitochondrial activity. The mitochondrial activity of the cells is reflected by the reduction of soluble yellow tetrazolium salt to insoluble purple formazan crystals. Only live cells are able to take up the tetrazolium salt. The enzyme (mitochondrial dehydrogenase) present in the mitochondria of the live cells is able to convert internalized tetrazolium salt to formazan crystals,

which are purple in colour. Then the cells were lysed and dissolved in DMSO solution. Any increase or decrease in viable cell number can be detected by measuring formazen concentration reflected in optical density determined in an ELISA reader at 570 nm.

MTT Assay: The cytotoxic activity of HepG2 cell lines was evaluated using nanoparticles by MTT assay as described previously by Hajiaghaalipour et al. [34]. Briefly, 100 µl of the cell suspension was seeded in a 96-well tissue culture plate (5000 cells/well) and incubated at 37°C for 24 hrs in a humi-dified 5% CO2 Incubator(New Brunswick). After 24 hrs cells were treated with different concentrations of nanoparticles and incubated for 48 hrs. After incubation, 10 µl (5 mg/mL in PBS) of MTT was added to each well and incubated for 4h at 37°C. The resulting formazan was dissolved in 100 µl of DMSO and the viable cells were determined by measuring the absorbance at 570 nm and 630 nm. The MTT containing medium was then discarded and the cells were washed with PBS (200 µl). The crystals were then dissolved by adding 100 µl of DMSO and this was mixed properly by pipetting up and down. Spectrophotometric absorbance of the purple blue formazan dye was measured in a microplate reader at 570 nm (Robonik ELISA analyser). Cytotoxicity was determined using Graph pad prim5 software.

3. RESULTS AND DISCUSSION

Cytotoxicity of SeNPs was evaluated on the HepG2 cell line by MTT assay. This assay assesses the mitochondrial activity of the viable cells by measuring its ability to reduce MTT into purple formazan crystals [35]. Fig. 1 illustrates the cytotoxicity of the biosynthesised SeNPs against HT-29 cell lines. The cytotoxicity of SeNPs was observed in a dose dependent manner where the viability was decreasing with increase in the concentration of nanoparticles. After treatment with SeNPs, 77% and 63% of cell viability were observed at 2 µg/ml and 4 µg/ml respectively. Whereas, 33.7% of cell viability was observed at 30µg/ml concentration against HepG2 cell line. The mechanism of cytotoxicity induced by nanoparticles can be any one of the following reasons. The production of ROS which can interrupt ATP synthesis and cause DNA damage, Secondly, by arrest of cell cycle arrest, angiogenesis and inhibition of tumour cell invasion. Our results clearly indicate that the synthesized SeNps have exhibited cytotoxic effects and careful safety studies are required considering the use of biogenic SeNPs synthesized nanoparticles.

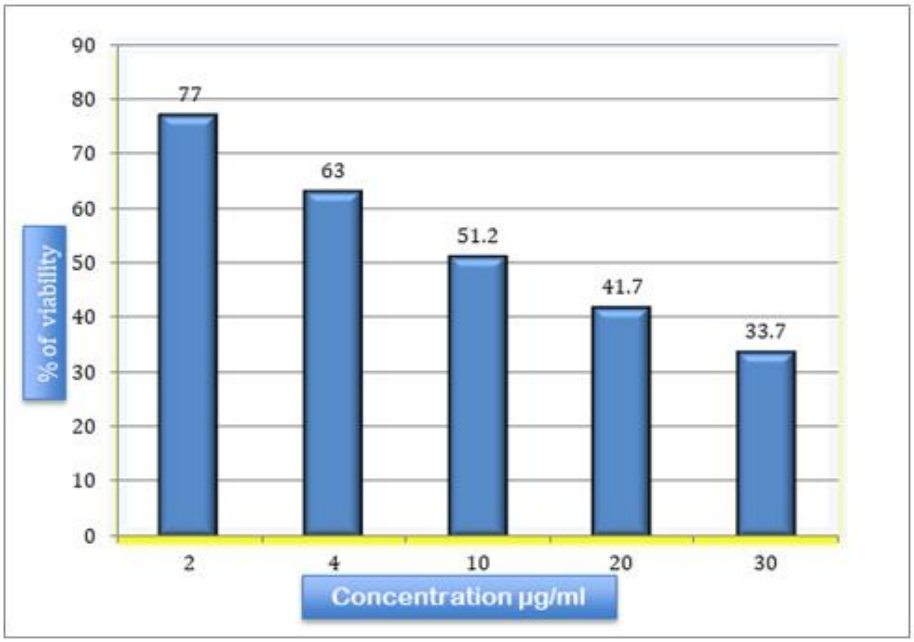

Fig. 1. MTT assay of the SeNPs on HepG2 cell lines. 77% and 63% of cell viability were observed at 2 µg/ml and 4 µg/ml respectively. Whereas, 33.7% of cell viability was observed at 30 µg/ml concentration

The development of Se nanoparticles and nanomaterials are a relatively new factor to be considered with respect to historical aspects of Se toxicity and environmental concerns [36]. The possible environmental effects, discharge rates and environmental levels of Se and Se-based nanoparticles such as CdSe need to be explored. As yet, no clear understanding of the toxicity of Se nanoparticles has been developed although nano-Se has been shown to affect glutathione S-transferase activity [14].

Initial studies on the toxicity of nano-Se to aquatic organisms have appeared in the literature. A comparison of the toxicity of nano-Se with sodium selenite was performed by evaluating the effects on Medaka fish after ten days exposure to selenite and Se nanoparticles at a dosage of 100 μg L^{-1} Se revealing that nano-Se had a greater toxicity due to hyper-accumulation [37]. A later study on larvae of the benthic aquatic midge, Chironomus dilutus, investigated the effects of dietary and waterborne Se-NPs on this common benthic invertebrate [38] which is frequently used as a test organism for assessing toxicity of sedimentary substances. The results of this study suggest that even the lowest Se(0) and SeNP concentrations tested (2.81 μg L^{-1} and 8.89 μg g^{-1} d.w. respectively), which were comparable to Se sedimentary levels in a lake polluted by uranium ore mining and milling, resulted in Se bioaccumulation mainly as SeMet. Inhibition of larval growth at higher concentrations due to both dietary and waterborne exposure was also observed [38].

Many studies reported the mechanism of how nanoparticles exhibit cytotoxicity by 1) the production of ROS thereby interrupting ATP synthesis and causing DNA damage, 2) by cell cycle arrest, angiogenesis and inhibition of tumour cell invasion [39,40]. The result clearly depicts that the synthesis SeNPs are cytotoxic to the HepG2 cell lines and can be effectively used as a chemotherapeutic drug.

4. CONCLUSION

The SeNPs also showed good anti-proliferative activity against the HepG2 cell line. These results suggest that further studies are required to know the exact mechanism involved in the cytotoxic activity against cell line HepG2 thereby permitting the SeNPs as a chemotherapeutic agent.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Available:https://doi.org/10.1016/j.jfda.2014.01.005

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/59701