Journal of Carcinogenesis
Covering Genesis to Therapeutics

Journal of Carcinogenesis, ISSN: 1477-3163
2024; Vol 23: Issue 1, https://carcinogenesis.com/

Green Production of Selenium Nanoparticles Integrated Hesperidin Nanoformulation and its Antioxidant, Anti-inflammatory, Antimicrobial and Embryonic Toxicology Assays

M.Suriya[1], J Kalaimathi[1]*, S. Revathi[2], R. Karthika[3], J.Deena Mol [4], K. Suresh[5], S.Rajeshkumar[6]

[1]Ph.D Research Scholar, PG & Research Department of Biochemistry, Theivanai Ammal College for Women (Autonomous), Affiliated to Annamalai University, Villupuram, Tamilnadu, India.

[1]*Head and Associate professor, PG & Research Department of Biochemistry, Theivanai Ammal College for Women (Autonomous), Affiliated to Annamalai University, Villupuram, Tamilnadu, India.

[2,3,4]Ph.D Research Scholar, PG & Research Department of Biochemistry, Theivanai Ammal College for Women (Autonomous), Affiliated to Annamalai University, Villupuram, Tamilnadu, India.

[5]Associate Professor, Department of Biotechnology & Biochemistry, Annamalai University, Chidambaram.

[6]Professor, Nanobiomedicine Lab, Centre for Global Health Research, Saveetha Medical College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Chennai, India.

Corresponding Author:

Dr. J. Kalaimathi.

Head and Associate professor, Dean of Research,Department of Biochemistry,Affiliated to Annamalai University,Theivanai Ammal College for Women (Autonomous), Villupuram

ABSTRACT

Introduction; In the biomedical field, selenium nanoparticles (SeNPs) have earned popularity since they possess strong antioxidant, anti-inflammatory, and antibacterial characteristics. Use of plants to synthesize SeNPs is ecofriendly and easily biocompatible, and does not require toxic chemicals. This paper is dedicated to the synthesis of SeNPs with the help of lining *Withania somnifera* and *Linum usitatissimum* extracts and their implementation into a nanoformulation consisting of hesperidin (NCs). The aim was to investigate the anti-inflammatory, antioxidant, antimicrobial and embryonic toxicity activity of SeNP-hesperidin nanoformulation.

Materials and Methods: he synthesis of SeNPs was conducted after combining an aqueous plant extract with sodium selenate and allowed to stir continuously over 48 hours. The UV-visible spectroscopy was used to confirm the formation of nanoparticles. The nanoformulation was prepared by mixing SeNPs and hesperidin, as well as sonication to provide complete mixing. The antimicrobial activity was measured with the help of the agar well diffusion method and the time-kill curve assay.Anti-inflammatory potential was evaluated through bovine serum albumin (BSA) denaturation, egg albumin denaturation, and membrane stabilization assays. Antioxidant activity was measured using DPPH, H_2O_2 scavenging, FRAP, ABTS, and nitric oxide scavenging assays. Biocompatibility was determined using the brine shrimp lethality assay and zebrafish embryonic toxicity evaluation.

Results :UV-visible spectroscopy confirmed SeNP formation at 395 nm. The nanoformulation exhibited significant antimicrobial activity, with *Candida albicans* showing the highest inhibition zone (20 mm at 100 µg/mL). Concentration dependent inhibition of protein denaturation, reaching 85% at 50 µg was observed at anti-inflammatory assays. Antioxidant assays revealed strong free radical scavenging activity, comparable to standard antioxidants. Cytotoxicity tests showed minimal toxicity at lower concentrations, while embryonic toxicity assays indicated a reduction in hatching rates at higher doses (40–80 µg/mL).

Discussion: The study highlights the synergistic effects of SeNPs and hesperidin, which enhanceanti-inflammatory, antimicrobial, and antioxidant properties. The formulation's selective antimicrobial activity suggests its potential for treating fungal and bacterial infections. It is biocompatible at lower concentrations implying that it can be used as a therapeutic agent. Nevertheless, the dose needs to be optimized in order to avoid embryonic toxicity at high doses.

Conclusion: SeNPs-hesperidin Nano- formulation was produced via the green technique and was highly bioactive with less toxicity over low doses. It is also a possible target of biomedical use due to its advantageous antimicrobial, antioxidant, and anti-inflammatory properties that could be used in wound healing, oxidative stress and infection inhibition. The path forward in the area of research must be concerned with formulation optimization, in vivo and clinical translation..

© 2024 Journal of Carcinogenesis | Published for Carcinogenesis Press by Wolters Kluwer-Medknow

Keywords: *Green synthesis, Selenium nanoparticles, Hesperidin nanoformulation, Biomedical applications.*

How to Cite: M.Suriya1, J Kalaimathi, S. Revathi, R. Karthika, J.Deena Mol , K. Suresh, S.Rajeshkumar, (2024) Green Production of Selenium Nanoparticles Integrated Hesperidin Nanoformulation and its Antioxidant, Anti-inflammatory, Antimicrobial and Embryonic Toxicology Assays, *Journal of Carcinogenesis*, *Vol.23, No.1*, 406-420

1. INTRODUCTION

Nanotechnology can be described as an interdisciplinary form of research since it allows the physical handling of materials at nanoscale level, which presents multi-faceted benefits in biomedical applications, such as, diagnostics, therapeutics, and delivery systems(1,2). Targeted drug delivery and personalised medicine have a long way to go due to the unique characteristics of nanomaterials their greater surface area, greater reactivity and better bioavailability(3). SeNPs are one of the nanomaterials that have attracted interest as they possess each of the antioxidant, anti-inflammatory, and antimicrobial properties and thus would be a promising representative as a biomedical application(4,5).

Selenium (Se) is a physiologically important trace element that carries out very important biological roles, especially in the regulation of oxidative stress and the modulation of the immune response(6). SeNPs have the advantage of combination of high bioactivity and low toxicity compared to conventional selenium supplements(7). They contain reactive oxygen species scavengers so they counter the reactive oxygen species helping prevent cancer and other degenerative diseases. Also, SeNPs have high antimicrobial properties and thus they damage microbial cell membranes and disturb cellular metabolism, proving toxic to several microbial and fungal pathogens(8). Moreover, SeNPs regulate the inflammation process, which can help decrease the level of chronic diseases. Such multifunctional activities make SeNP a possible candidate in the wound healing, infection control and inflammation-associated disorders(9,10).

The reduction and stabilization of plant extracts as eco-friendly and green routes to synthesize SeNPs are highly noticed. The advantages of green synthesis approaches have been outlined to be eco-friendly in that the toxicity of chemicals in the product is eliminated, biocompatible that enhances the nanoparticles with respect to biomedical uses and cost effective because the plant materials reduce cost of production(11,12). This paper reports the use of *Withania somnifera* (Ashwagandha) and *Linum usitatissimum* (Flaxseed) as bio-reductants to synthesize SeNP. *Withania somnifera* can be used in the form of with anolides and alkaloids, which are characterized by antioxidant and anti-inflammatory effects, and alkalines *Linum usitatissimum* also contains lignans and omega-3 fatty acids, which help stabilize nanoparticles and increase their ability to help(13).

Bioactive flavonoids like Hesperidin isolated from citrus fruits are recognized as effective antioxidant, anti-inflammatory and antibacterial agents, thus a good choice against integration in the SeNP nanoformulation(14). It is predicted that the combination would lead to synergetic therapeutic benefits, including a broader antioxidant potential by boosting the ROS scavenging activity, extended anti-inflammatory activity when suppressing crucial inflammatory mediators, and an amplified antimicrobial capacity in terms of microbial membrane disruption and prevention of biofilms formation(15,16). Also, hesperidin enhances the solubility and stability of SeNPs resulting in enhanced bioavailability and enhanced medicinal effects. The contribution of the hesperidin in combination with SeNPs aims to improve the functionalities of current products to have greater plant therapeutic potential and have the least toxicity if used medically(17).

The major objective of the research is to produce SeNPs via the green synthesis technique and integrate them into a nano formulation of hesperidin, and test its antioxidant, anti-inflammatory, anti-microbial, and embryonic toxicity characteristics (18). The specific aims involve the ultraviolet visible spectroscopy characterization of the synthesized SeNPs, the determination of the antioxidant activities as per the ferric reducing antioxidant power (FRAP), 1, 1-diphenyl -2-picryl -hydrazyl(DPPH), 2, 2 azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS),hydrogen peroxide (H2O2).

This study will allow developing a new nanoformulation that has stronger therapeutic characteristics and better biocompatibility. The green synthesis procedure follows the increase in interest to sustainable nanotechnology, and it provides an environmentally friendly, inexpensive, and safe option to be used in biomedical purposes (20).

2. MATERIALS AND METHODS:

Preparation of Plant Extract:

The plant extract was prepared by exact measurements of 1 g of *Withania somnifera* powder and *Linum usitatissimum* was added to 100mL of distilled water. Having combined the mixture, it was then treated with controlled thermal extraction process by heating it over a heating mantle at a temperature range of 60-70ºC of liquid near about 15-20 minutes with a view of improving the release of bioactive components. After the extraction, the mixture was carefully filtered over Whatman No. 1 filter paper to remove the undissolved particulates to ensure clear liquid. The aqueous extract that we

© 2024 Journal of Carcinogenesis | Published for Carcinogenesis Press by Wolters Kluwer-Medknow

received, containing phytoconstituents of the two botanical sources, has been kept in low temperature refrigeration with the view of preserving hybridization and bioactivity of the chemical substance which in turn could be utilized in the process involving green synthesis of selenium nanoparticles.

Figure 1: Preparation of aqueous extract from *Withania somnifera* and *Linum usitatissimum* for SeNP synthesis. (A–B) Weighing 1 g each of plant powders; (C) Mixing in 100 mL distilled water; (D) Heating at 60–70°C for 15–20 min; (E) Filtration using Whatman No. 1 paper; (F) Clear extract stored for SeNP synthesis.

Preparation of SeNPs:

30 mM sodium selenate solution was prepared in 50 mL of distilled water and mixed with 50 mL of *Withania somnifera* and *Linum usitatissimum* extract to initiate the green synthesis of selenium nanoparticles SeNPs. The reaction was stirred at 600 rpm for 24–48 hours at room temperature. UV–Visible spectrophotometer was used to observe the synthesis progress at regular time intervals. After completion, the SeNPs were separated by centrifugation at 8000 rpm for 10 minutes. The resulting pellet was collected and stored in airtight Eppendorf tubes for further characterization, While the Supernant was discard to ensure sample purity.

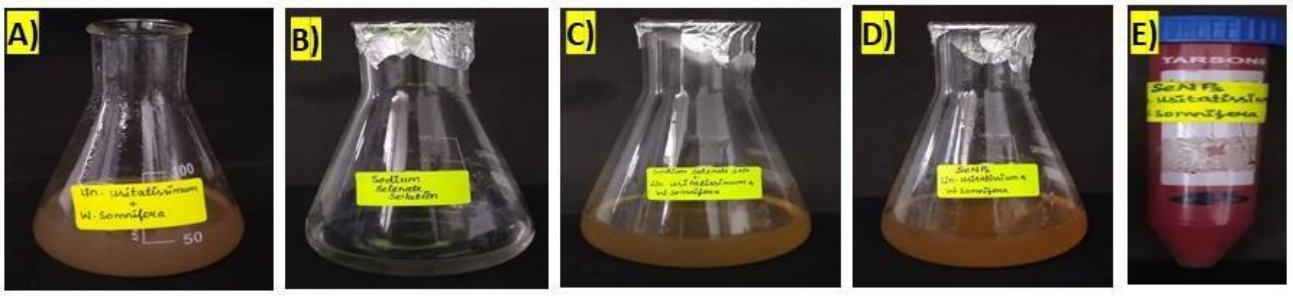

Figure 2: Green synthesis of SeNPs using *Withania somnifera* and *Linum usitatissimum* extract. (A) Prepared plant extract; (B) Sodium selenate solution; (C) Mixing of precursor with extract to initiate synthesis; (D) Color change indicating SeNP formation; (E) SeNPs pellet obtained after centrifugation.

Preparation of Nanoformulation

The nanoformulation was prepared by dissolving 100 mg of hesperidin in 1 mL of DMSO, followed by dilution with 4 mL of PBS to a final volume of 5 mL. The solution was agitated for 1 hour to ensure uniform mixing. An equal volume (1 mL) of this hesperidin solution was then combined with 1 mL of selenium nanoparticle (SeNP) suspension. The mixture was sonicated for 30 minutes to achieve stable and homogeneous incorporation of hesperidin into the SeNP matrix, enhancing dispersion and formulation stability for biomedical applications.

© 2024 Journal of Carcinogenesis | Published for Carcinogenesis Press by Wolters Kluwer-Medknow

Figure 3: Preparation of hesperidin–SeNP nanoformulation. (A) Hesperidin powder; (B) Preparation of stock solution; (C) Incorporation of SeNPs with hesperidin, indicated by a distinct color change.

In vitro assays

The biological evaluation of selenium nanoparticles (SeNPs) incorporated with hesperidin nanoformulation was carried out through multiple in vitro assays. Antimicrobial efficacy was determined using the agar well diffusion method on Muller-Hinton Agar (MHA) plates seeded with *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, and *Pseudomonas* sp. 9 mm diameter wells were filled with 100 µL of the nanoformulation at concentrations of 25, 50, and 100 µg/mL, alongside a commercial antibiotic (positive control) and distilled water (negative control). After incubation at 37°C for 24 hours, zones of inhibition were measured to assess antimicrobial activity. The time-kill curve assay evaluated bactericidal and fungicidal kinetics using microbial inoculums (10^6 CFU/mL) treated with the nanoformulation at the same concentrations. Samples were collected at intervals from 0 to 5 hours, plated, and CFU/mL was quantified after 24 hours of incubation to determine time-dependent microbial reduction. Antioxidant potential was assessed via hydrogen peroxide scavenging, DPPH, ABTS, FRAP, and nitric oxide scavenging assays as per the method of Dathan et al. (2025). Similarly, anti-inflammatory activity was examined using egg albumin denaturation,BSA denaturation, and membrane stabilization assays following Dathan et al. (2025). Cytotoxicity was tested using the Brine Shrimp Lethality Assay (BSLA), where *Artemia salina* nauplii were exposed to SeNP-hesperidin nanoformulation at concentrations of 5–80 µg/mL, and survival was recorded at 24 and 48 hours. High biocompatibility was indicated by low mortality at lower doses. Embryonic toxicity was evaluated using zebrafish (*Danio rerio*) embryos, following the methodology of Tharani et al. (2023), to assess the formulation's developmental safety. These comprehensive analyses confirmed the SeNP-hesperidin nanoformulation's promising biomedical properties.

3. RESULT:

UV-Visible Spectroscopy

In figure 4, UV spectra was observed to monitor the synthesis and formation of SeNPs by keeping specific time intervals. It demonstrates the UV-visible spectra of nanoparticles where characteristic absorption peak (395nm) of SeNPs indicate the effective nanoparticle formation. A moderate peak assigned to SeNPs at 36 hours with the peak of around 1.5 AU indicates the possible initial phase of nanoparticle formation. At 48 hours of incubation, the absorbance peak climbed up to approximately 2.0 AU, which means that the nanoparticles concentration improved and became more stable with time. The graded absorbance increases 36-48 hours shows that the production of good yield and the stabilization of the nanoparticles was due to the long hour of stirring. The absorption spectrum was solid and there were no extra uncoupled peaks when using 350 to 650 nm to confirm the purity and reproducibility of the SeNPs synthesized with the use of Withania somnifera and Linum usitatissimum extracts. These findings favor the prospect of the green synthesis technique into producing stable and reproducible selenium nanoparticle to continue with further application.

© 2024 Journal of Carcinogenesis | Published for Carcinogenesis Press by Wolters Kluwer-Medknow

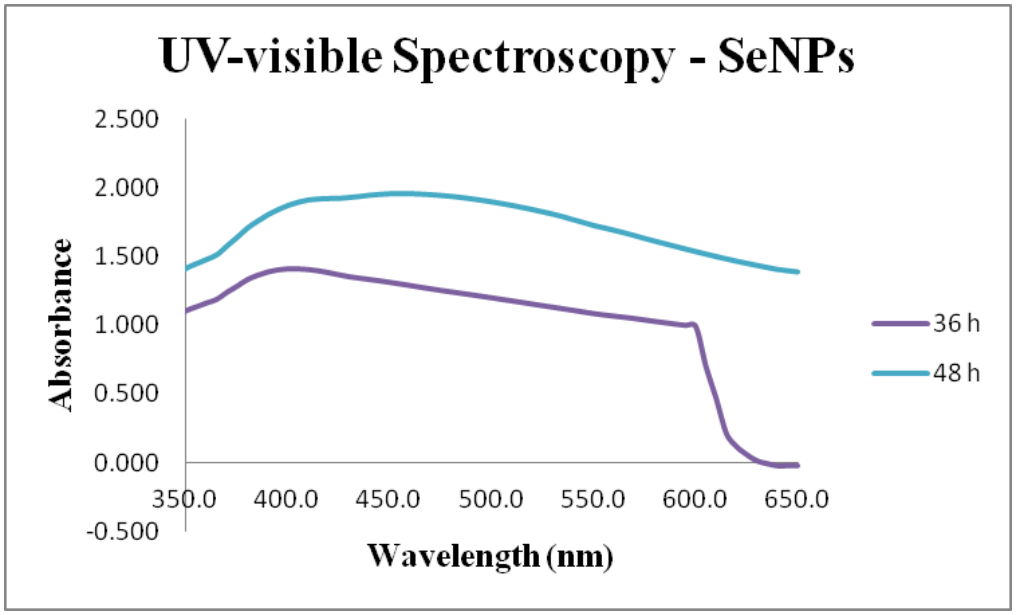

Figure 4: Ultra violet- Visible spectra of green synthesized SeNPs nanoformulation

Antimicrobial Activity

The antimicrobial efficacy of the SeNP-hesperidin nanoformulation (NCs) was evaluated against *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, and *Pseudomonas sp.* using the agar well diffusion method. The inhibition zones were measured at concentrations of 25, 50, and 100 µg/mL, with a control included for comparison (Figures 5 and 6).

The results indicate a concentration-dependent increase in antimicrobial activity across all tested microorganisms. *Candida albicans* exhibited the highest sensitivity to the SeNP-hesperidin NCs, with inhibition zones measuring 12 mm, 14 mm, and 20 mm at 25, 50, and 100 µg/mL, respectively. *Escherichia coli* showed inhibition zones of 10 mm, 13 mm, and 16 mm at increasing concentrations, while *Staphylococcus aureus* exhibited zones of 12 mm, 13 mm, and 15 mm. In contrast, *Pseudomonas sp.* displayed a consistent inhibition zone of 9 mm at all tested concentrations, indicating lower sensitivity to the nanoformulation.

These findings suggest that the SeNP-hesperidin NCs possess effective antimicrobial properties, particularly against *C. albicans*, followed by *E. coli* and *S. aureus*, with a lesser effect on *Pseudomonas sp.*. This differential sensitivity highlights the potential use of the SeNPs-hesperidin formulation as an antimicrobial agent, especially for applications targeting fungal and bacterial infections.

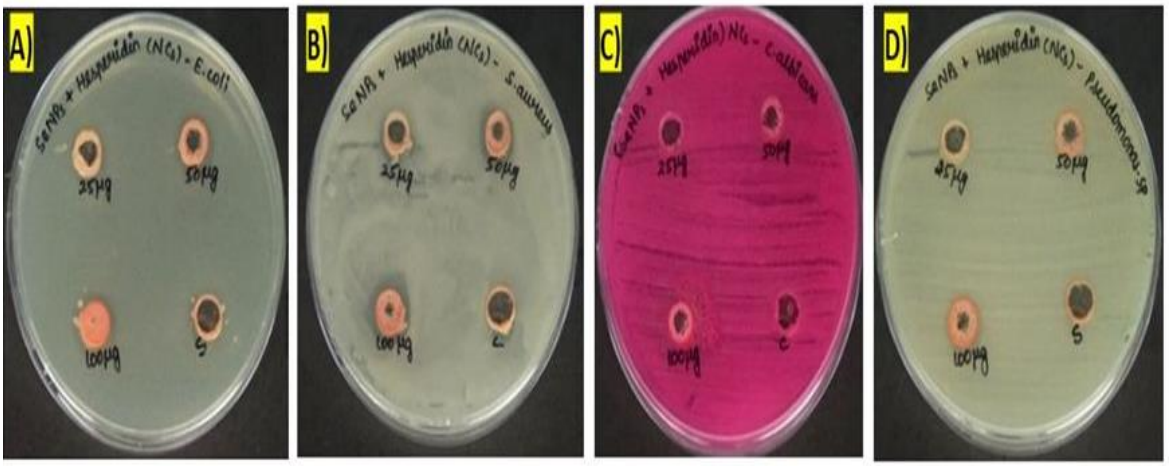

Figure 5: Agar well diffusion method on the effect of SeNPs incorporated with hesperidin

© 2024 Journal of Carcinogenesis | Published for Carcinogenesis Press by Wolters Kluwer-Medknow

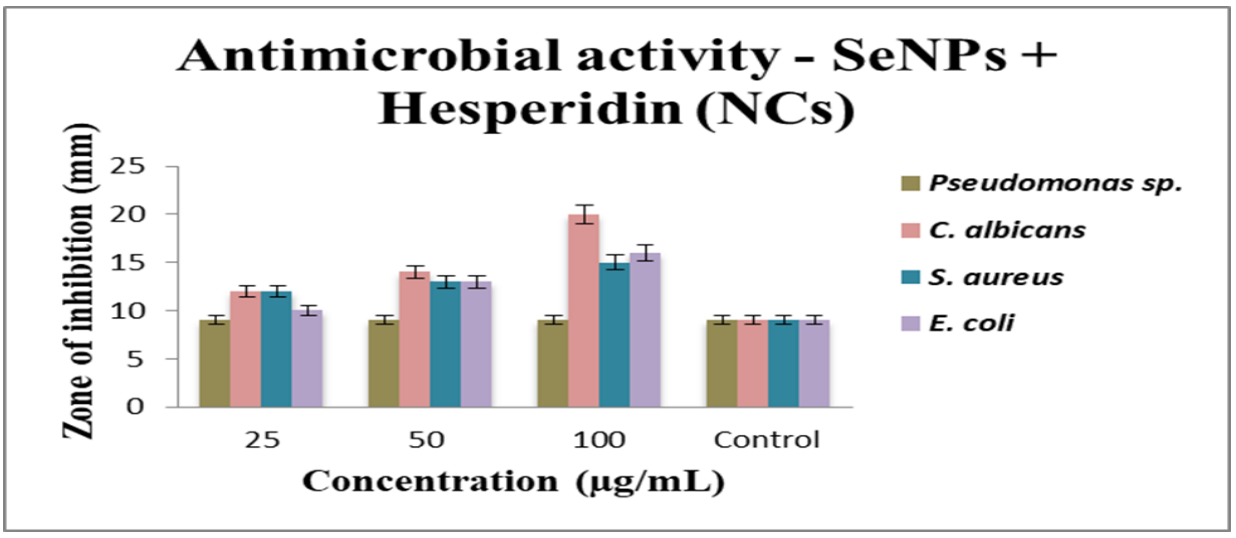

Figure 6: Antimicrobial activity results of SeNPs incorporated with hesperidin against wound pathogens

Time-Kill Curve Assay

The antimicrobial activity of SeNPs incorporated with hesperidin was assessed using a time-kill curve assay against *Pseudomonas* sp., *Candida albicans*, *Staphylococcus aureus*, and *Escherichia coli* over 4 hours at concentrations of 25, 50, and 100 µg/mL (Figure 7A–D).

For *Pseudomonas* sp., CFU/mL values remained stable across all concentrations, indicating minimal antibacterial effect. In contrast, *C. albicans* showed a notable CFU reduction at 100 µg/mL and moderate activity at 50 µg/mL, suggesting effective antifungal properties at higher doses.

Against *S. aureus*, the nanoformulation demonstrated a gradual and concentration-dependent decrease in CFU/mL, with the strongest effect at 100 µg/mL. A similar trend was observed for *E. coli*, where the highest concentration showed significant bacterial reduction, and moderate activity was seen at 50 µg/mL.

Overall, the SeNP–hesperidin nanoformulation displayed dose-dependent antimicrobial effects, particularly against *C. albicans*, *S. aureus*, and *E. coli*, while being less effective against *Pseudomonas* sp., highlighting its potential as a selective antimicrobial agent.

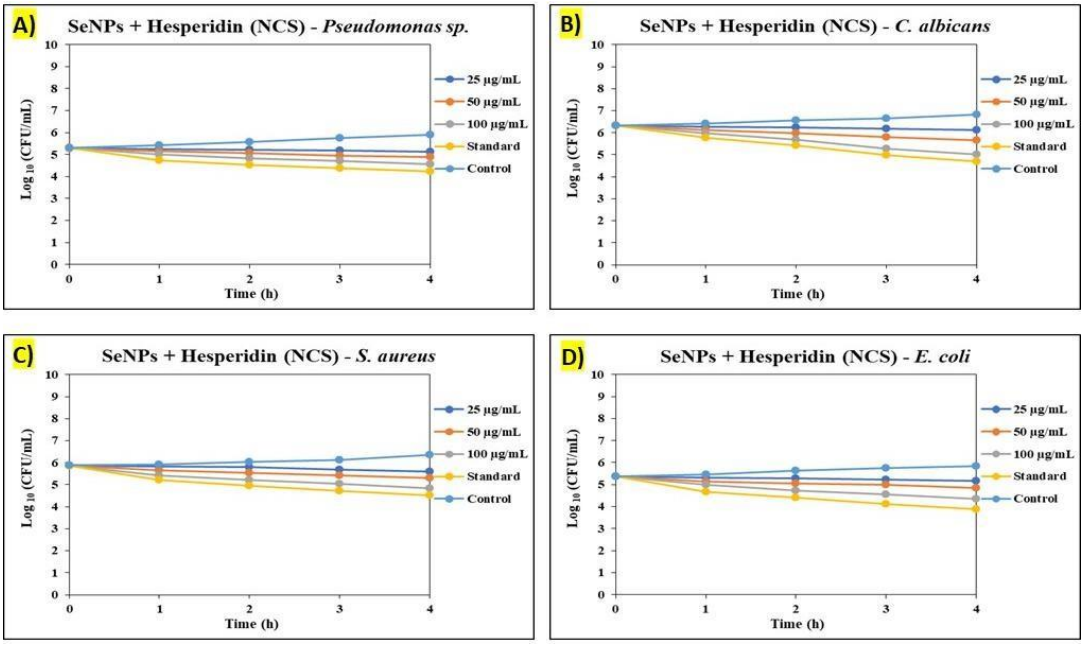

Figure 7: Time kill curve assay of SeNPs incorporated with hesperidin *Pseudomonas* sp. (A), *C.albicans* (B), *S.aureus* (C), and *E.coli* (D)

© 2024 Journal of Carcinogenesis | Published for Carcinogenesis Press by Wolters Kluwer-Medknow

Anti-inflammatory Activity

The anti-inflammatory activity of SeNPs incorporated with hesperidin was evaluated using BSA denaturation, EA denaturation, and membrane stabilization assays at concentrations of 10–50 µg/mL, compared to a standard agent (Figure 8).

In the BSA assay, the nanoformulation showed a concentration-dependent inhibition of protein denaturation, increasing from ~55% at 10 µg/mL to ~85% at 50 µg/mL, closely matching the standard's efficacy. Similarly, in the EA denaturation assay, inhibition rose from ~50% to over 80% across the tested concentrations, indicating consistent anti-inflammatory potential.

The membrane stabilization assay further supported these findings, with inhibition starting at ~60% and increasing to ~85% at the highest dose, reflecting strong protection against cell membrane lysis.

Collectively, these results confirm the SeNP–hesperidin nanoformulation's robust, dose-dependent anti-inflammatory activity, comparable to the standard and suggesting its promise as a natural therapeutic agent.

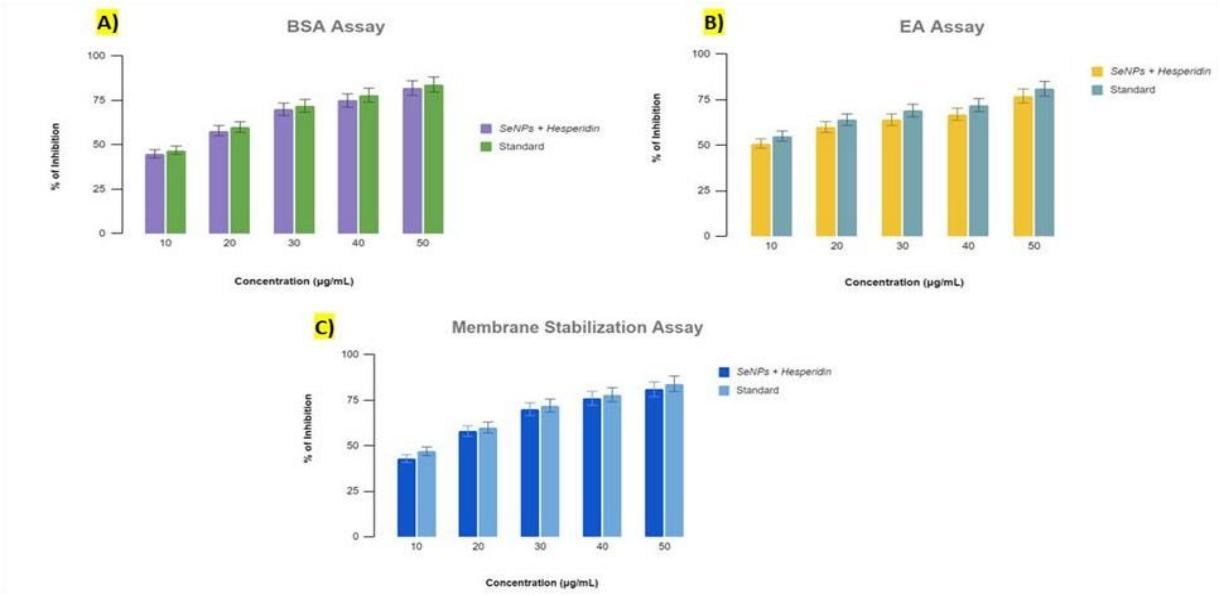

Figure 8:Anti-inflammatory potential of SeNPs incorporated with hesperidin

Antioxidant activity

The antioxidant activity of SeNPs incorporated with hesperidin was assessed using DPPH, H_2O_2, FRAP, ABTS, and nitric oxide scavenging assays at concentrations ranging from 10 to 50 µg/mL, compared with a standard antioxidant (Figure 9).

In the DPPH assay, the nanoformulation exhibited strong concentration-dependent radical scavenging, starting at ~70% inhibition at 10 µg/mL and reaching ~85% at higher doses, comparable to the standard. Similar trends were observed in the H_2O_2 scavenging assay, where inhibition rose from ~55% at 10 µg/mL to ~85% at 50 µg/mL, indicating efficient peroxide neutralization.

The FRAP assay showed notable ferric reducing power across all concentrations, beginning at ~75% inhibition at 10 µg/mL and reaching ~85% at higher doses, closely aligning with the reference antioxidant. In the ABTS assay, inhibition increased from ~73% at the lowest dose to ~85% at the highest, confirming substantial antioxidant potential. Nitric oxide scavenging followed the same dose-dependent pattern, with inhibition ranging from ~72% to 85% across concentrations.

Overall, the SeNP–hesperidin nanoformulation demonstrated consistent and potent antioxidant activity in all assays, highlighting its potential as a strong free radical scavenger.

© 2024 Journal of Carcinogenesis | Published for Carcinogenesis Press by Wolters Kluwer-Medknow

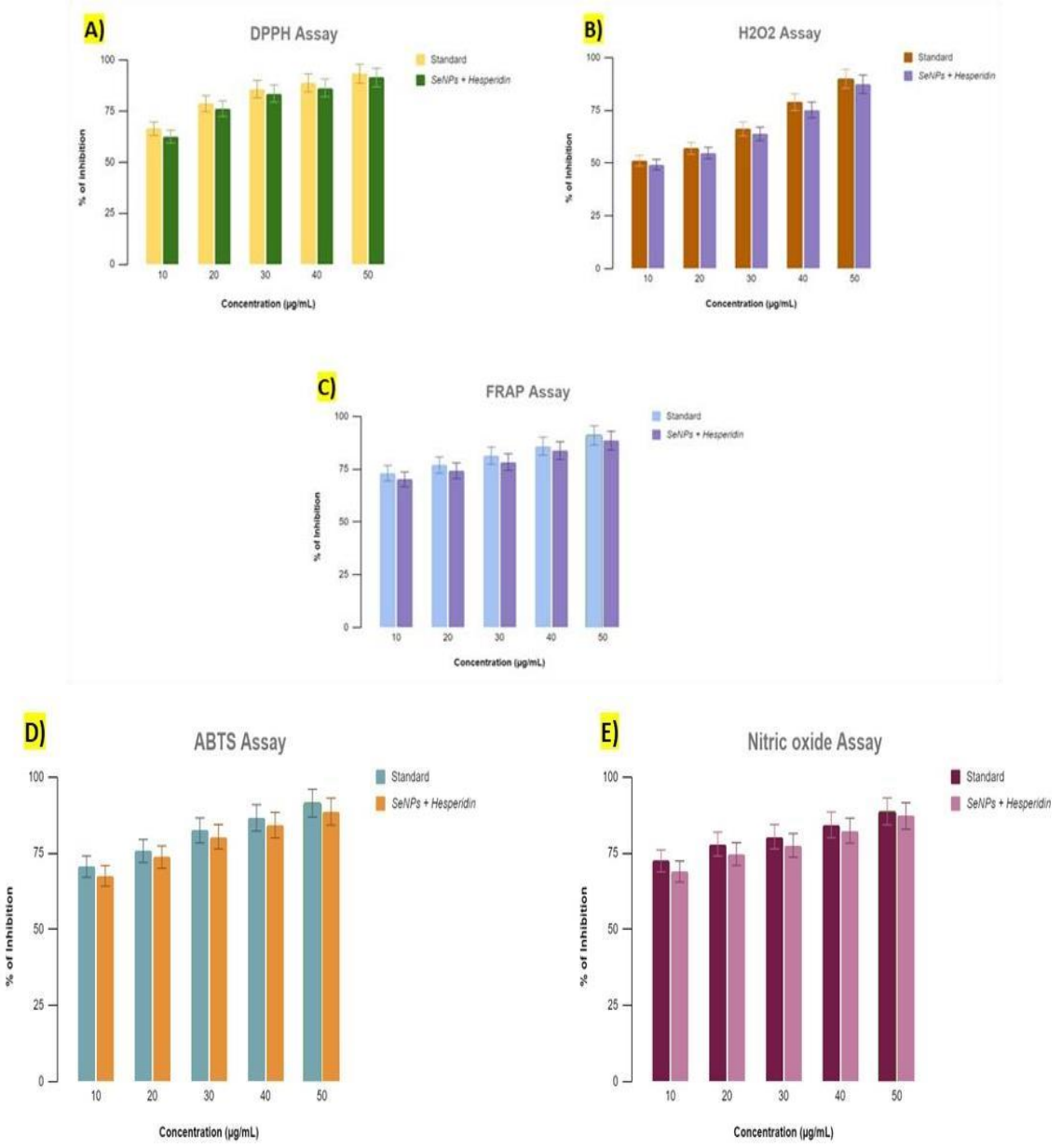

Figure 9: In vitro assays used to assess the antioxidant potential of selenium nanoparticles SeNPs incorporated with hesperidin

Cytotoxicity of SeNPs

A brine shrimp lethality test was used in determining the cytotoxic dose of the SeNPs integrated with hesperidin nanoformulation (NFs) at diluted concentrations of 5, 10, 20, 40 and 80 µg/mL over two days. Each concentration was noted and then a percentage of live nauplii as assessed against a control group is recorded (Figure 10). At Day 1, no significant cytotoxic effect was observed at all concentrations given as nauplii viability was high with survival near-100 percent. Likewise on Day 2, the viability was still higher than 95 percent on all of the tested concentrations, with no significant drop of the live nauplii in comparison to the control. Based on such findings, the SeNP-hesperidin nanoformulation can be considered highly biocompatible because, despite the two days of observation, no cytotoxicity could be observed within reasonable concentrations. On the whole, the fact that the percentage of live nauplii at both time points is high can be seen as evidence that the SeNP-hesperidin nanoformulation can be used as a safe material in the future in the biomedical sector.

© 2024 Journal of Carcinogenesis | Published for Carcinogenesis Press by Wolters Kluwer-Medknow

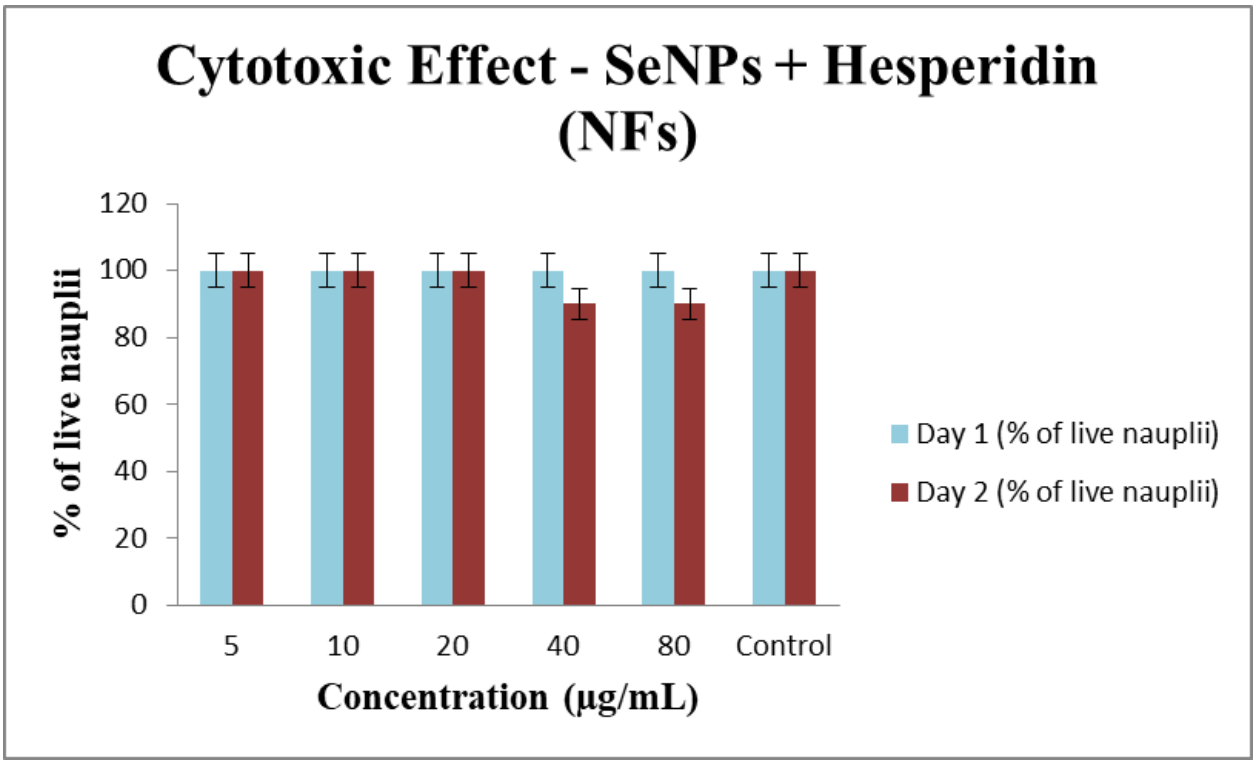

Figure 10: Brine shrimp lethality assay of green synthesized SeNPs + Hespiridin Nanoformulation

Embryonic Toxicity Evaluation:

An analysis of the effects of SeNPs embodied in hesperidin nanoformulation was done by observing the zebrafish embryos at different developmental stages. In figure 11, development proceeds in a normal fashion through 3 stages of prominence; as A) early blastodisc formation, B) segmentation to intact yolk sac structure and C) the larval stage with prominent morphological characteristics including development of eyes and tail. These results show that when the concentrations of the nanoformulation (seNP-hesperidin) were subjected to low doses into embryos, the embryos grew normally with no morphological deformations. This further implies that the nanoformulation is not toxic to vital developmental processes, and encourages further usage as a conversational agent in bio-medical applications.

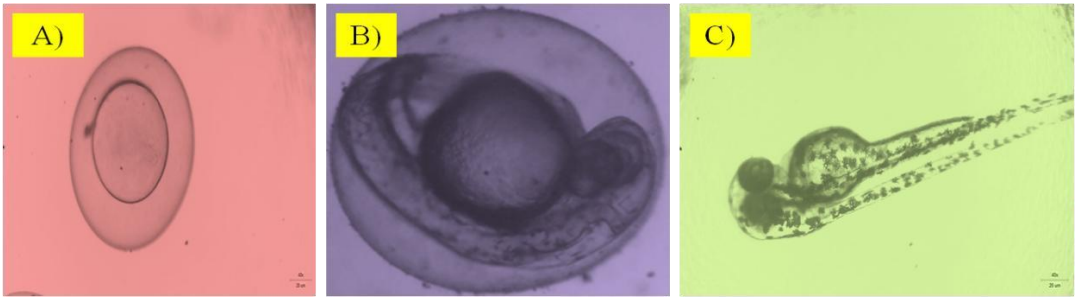

Figure 11: Microscopic images of zebrafish embryos exposed to SeNPs–hesperidin nanoformulation. (A) Early embryonic stage, (B) Segmentation stage, (C) Larval stage with normal morphology, indicating no developmental toxicity at low concentrations.

Embryo mortality due to the SeNPs combined with hesperidin nanoformulation (NF) was observed by examining the hatchability of the embryos at different dosages (5, 10, 20, 40, and 80 ug/ml). hatchability of embryos was also monitored in the group of control (Fig. 12). When tested at lower concentrations (5, 10, and 20 ug/mL), the rate of hatching was also high (100 percent) meaning that there was no significant toxicity to the embryos at these levels. Nevertheless, a significant decrease in the hatching rate was reported at greater concentration where hatching rate was reduced to about 75% at 40

© 2024 Journal of Carcinogenesis | Published for Carcinogenesis Press by Wolters Kluwer-Medknow

ug/ml to about 60% at 80 ug/ml. A control group yielded a hatching percentage of close to 100%, which shows the formulation of SeNP in combination with hesperidin affected embryonic development, which was concentration-dependent. Such findings indicate that in low doses the SeNP-hesperidin nanoformulation is biocompatible, whereas higher doses can affect embryonic hatching and hence there should be a limit of safe use. This nonlinear dose-effect implies that nanoparticle dose optimization is pivotal in possible biomedical and environmental areas.

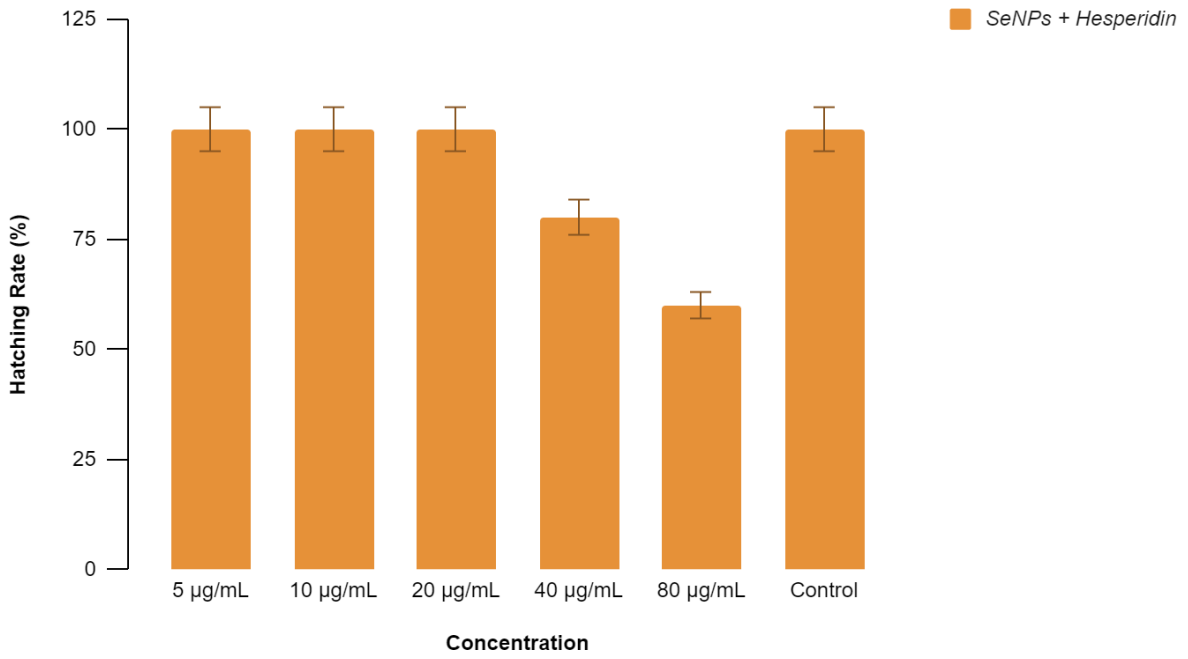

Figure 12: Effect of SeNPs incorporated with hesperidin on the hatching rate of zebrafish embryos

To identify the possible toxicity, the viability rate of the zebrafish embryos exposed to different concentrations (5, 10, 20, 40 and 80 µg/mL) of theSeNPs with hesperidin nanoformulation (NF) was evaluated (Figure 13). Embryo viability was nearly 100% at the concentration of 5, 10, and 20 µg/mL, implying minimal or no toxic effects. Nevertheless, the viability rate was of high concentrations at 40 and 80µg/mL being approximately 80 and 75 % respectively. The viability rate of the control group was exactly close to 100 percent, which stressed a dose-dependent reduction in viability with dramatic concentration of SeNP-hesperidin composition. This outcome implies that although the embryos tolerate low levels of the SeNP-hesperidin nanoformulation, they show medium toxicity in high concentrations which can be deduced through the decreased viability of embryos. This effect is concentration-dependent which highlights the requirement of regulated dosage in possible biomedical and environmental uses of the nanoformulation.

© 2024 Journal of Carcinogenesis | Published for Carcinogenesis Press by Wolters Kluwer-Medknow

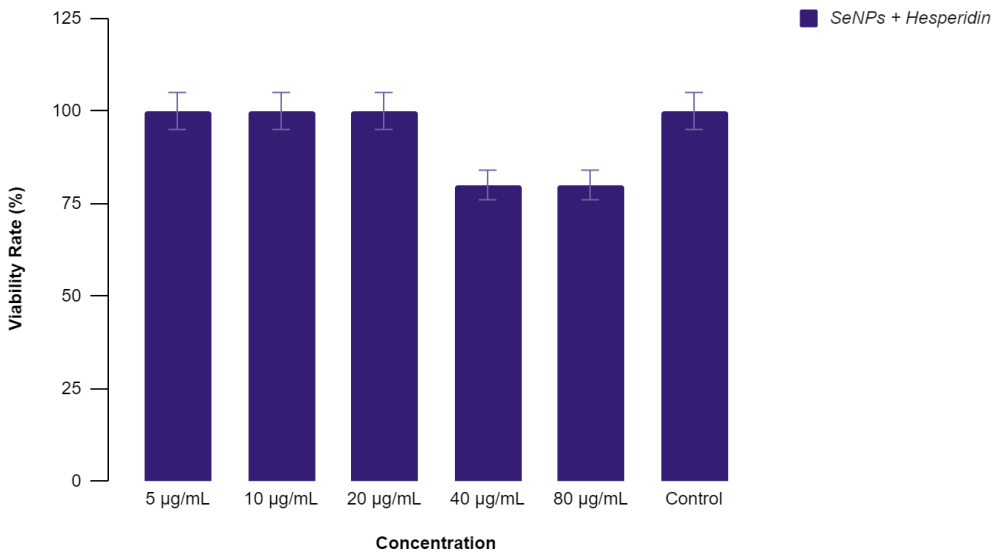

Figure 13: Effect of SeNPs incorporated with hesperidin on the viability rate of zebrafish embryos

4. DISCUSSION:

The present study reveals the ability of Withania somnifera and Linum usitatissimum extracts to help generate SeNPs successfully, which can be further entrapped into hesperidin-based nanoformulation (NCs). Like it was done in the UV-visible spectroscopy analysis, the aspect of formation of SeNPs was visualized with a distinct absorption peak of 395 nm that described the formation of stable nanoparticle(23). The fact that the absorbance that was gradually increasing over a time period of 36 hours to 48 hours showed that there was a definite gradual process of formation of the nanoparticles which was made possible by the phytochemicals which present in the plant extracts(24). The synthesis yielded no secondary peaks in the range 350-650nm defining the purity and stability of the synthesized SeNPs(25). These findings show that this technology of nanoparticle production with the help of plants has the potential to become a green replacement to the conventional chemical manufacturing methods of nanoparticles that ensures compatibility and sustainability towards its biomedical application(26,27).

The extent of antimicrobial potential of SeNP-hesperidin nanoformulation was carried out through agar well diffusion and time-kill experiment. These results showed that the trend of antimicrobial activity is concentration-dependent where the greatest inhibition zone was against Candida albicans (20 mm at 100 mg/mL) as compared to Escherichia coliand (28). On the other hand Pseudomonas sp. was highly resistant to the nanoformulation. The potent antifungal activity to the C. albicans can be attributed to the synergetic activity of SeNPs and hesperidin with antimicrobial activity(29). It has been disclosed that selenium as nanoparticles generate reactive oxygen speciesand the damage of microbial membranes. In addition, the use of hesperidin as a powerful antimicrobial flavonoid might also be the reason why the antifungal effect was enhanced by blocking ergosterol synthesis and biofilm formation (30,31).

The presence of a time-kill curve also determined that the SeNP-hesperidin formulation could reduce the CFU/mL efficiency with time, in particular against C. albicans, S. aureus, and E. coli (32). However, the low activity to Pseudomonas sp suggests that further optimisation steps such as combination therapy is required to enhance the performance of the agent towards gram-negative ATCC(33,34).

Nanoformulation of SeNP-hesperidin had anti-inflammatory activity and the assay was measured by the amount of bovine serum albumin (BSA) denaturation, egg albumin denaturation and membrane stabilization. The formulation has good dose response anti-inflammatory effect with approximately 85 percent inhibition at 50 ug/ml in all assays(35). This restriction of denaturation of the proteins signifies that the nanoformulation possesses the capacity of inhibition of inflammatory reactions at the molecular level(36). It also presented sufficient strength to hemoprotect red blood cells as explained by the membrane stabilization test and empower the cytoprotective properties of the compound. The high anti-inflammatory effect, though evident in this study, can be said to be present in both selenium nanoparticles and hesperidin(37). It has also been considered that selenium alters the inflammatory pathways by repressing the pro inflammatory cytokine production, whereas hesperidin has been argued to inhibit the cyclooxygenase (COX) and lipoxygenase (LOX) pathways which are important pathways of inflammation(38). The prevailing synergetic influence of co-incorporation of SeNPs and hesperidin enforces the perspectives of the nanoformulation as the natural anti-inflammatory product in treatment of the inflammation-related disorders(39,40).

To determine the antioxidant properties of the SeNP- hesperidin nanoformulation, various in vitro tests were carried out namely, DPPH, hydrogen peroxide scavenging (H2O2), ferric reducing antioxidant power (FRAP), and ABTS, and nitric oxide scavenging assays. The findings showed that the antioxidant activity increased with concentration and at 50 µg/mL the inhibition rates were approximated to be 85 percent close to the normal antioxidant agent(41). The DPPH radical scavenging experiment showed that SeNP-hesperidin had powerful free radicals scavenging effect, which is vital in limiting oxidative stress. In the same trend, in the hydrogen peroxide scavenging assay and FRAP assays, it was observed that the nanoformulation was better in neutralizing the ROS thus, preventing oxidative damage and degradation of cells(42). The strong antioxidant potential reflected by the results in all the tests indicates the possibility of using SeNP-hesperidin nanoformulation in abating the oxidative stress-related diseases. Its antioxidant quality is also boosted by the presence of hesperidin that is a known flavonoid compound which has high radical-scavenging abilities(30). These results imply that the SeNP-hesperidin nanoformulation may be used in the treatment of antioxidants on Neurodegenerative diseases, wound healing, and chronic inflammatory diseases(43).

Brine shrimp lethality assay which is a method showing the cytotoxicity of the SeNP-hesperidin nanoformulation indicated very little toxicity at all concentrations tested (5-80 ug/mL). The top nauplii survival levels (> 95%) on Day 1 and Day 2 show that nanoformulation has good safety profile and it did not show any considerable toxic effect at these concentration (44). The nanoformulation embryonic toxicity was also tested by using zebrafish models whose embryos were examined using microscopy to analyze normal blastodisc development, segmentation and larval morphology at low doses(30). Hatching rate was also high (5-20 ug/ml), but there was inhibition of hatching rate beyond this concentration (40-80 ug/ml) a dose dependent action on embryonic viability was observed (about 60-75%). The viability study of the embryo also revealed that the embryos subjected to the low doses of the nanoformulation had high survival rates as compared to those exposed to high doses of the nanoformulation which showed moderate cytotoxicity. These data emphasize the fact that the SeNP-hesperidin nanoformulation is biocompatible at lower dose, but the dose needs to be optimized to avoid any possible toxicity in the case of biomedical application(45).

In general, the current work proved that green synthesized SeNP-hesperidin nanoformulation had strong antioxidant, anti-inflammatory, and antimicrobial activity and had a satisfactory biocompatibility profile at low concentrations. Synergetic action between selenium nanoparticles and hesperidin moiety improves the therapeutic efficiency of the nano formulation, thus promising as a drug in several biomedical applications(46). This high antioxidant action of the formulation may have a potential in the treatment of oxidative stress induced diseases and the formulation can be used as an alternative to treat inflammation induced disease due to its anti-inflammatory qualities(47). Moreover, its antimicrobial activity, especially against fungal pathogens, indicates its possible application in antimicrobial coatings, wound dressings and infection control. Nevertheless, there is need to do more research on the optimization of the nanoformulation, it long-term stability and its pharmacokinetics in in vivo models. Research in the future is required to investigate its use in more progressive treatment fields on cancer treatments, targeted drug delivery and wound healing formulations(48).

5. CONCLUSION:

In this research, it was possible to show that selenium nanoparticle (SeNPs) synthesis was done using Withania somnifera and Linum usitatissimum extracts and subsequently introduced into a hesperidin-derived nanoformulation (NCs). This synthesized nanoformulation of SeNP-hesperidin was powerful in its antioxidant, anti-inflammatory, and antimicrobial powers and also it showed little cytotoxicity and lower levels of embryonic toxicity at low doses. The obtained SeNPs were sufficiently pure and stable in accordance with UV-visible spectroscopy and stored as such, whereas the broad-spectrum antimicrobial potential of the nanoformulation was exemplified by the agar well diffusion and time-kill curve studies, demonstrating the potency against Candida albicans, Escherichia coli and Staphylococcus aureus. The anti-inflammatory tests showed the protein denaturation and the membrane stabilization inhibition in a dose-dependent manner, which led to the potential in the treatment of inflammation. Also, the formulation demonstrated high free radical scavenging property reaffirming its ability as an antioxidant. The results of biocompatibility analysis showed that nanoformulation is safe to use in lower concentrations, but higher dosage could influence the hatching and viability rates using brine shrimp lethality assay and zebrafish embryonic toxicity test. These results indicate that SeNP-hesperidin NCs may be used in wound healing, antimicrobial coating, oxidative stress kind of treatment, or even inflammation management. The assessment of its long-time stability and pharmacokinetics is required to further its clinical application in the future, which also need following research on improvement of its formulation. The report underscores the therapeutic value of phytochemical-based nanotechnology in the biomedical fields.

6. ACKNOWLEDGEMENTS

The authors express their sincere gratitude to the Research Supervisor, Department of Biochemistry, Theivanai Ammal College for Women (Autonomous), Villupuram, for her invaluable guidance, encouragement and expertise throughout the study. Special appreciation is also extended to the research team at Saveetha Medical College, Chennai and Annamalai University, Chidambaram for their collaborative support and technical assistance.

7. AUTHORS CONTRIBUTIONS

MS conducted the laboratory experiments, collected the data and drafted the manuscript. SR, RK and DM were responsible for the investigation, methodology optimization and statistical analysis. JK, KS and SRK contributed to the study design, coordination and manuscript revision. All authors read and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest: The Authors declare no conflict of interest.

Ethical issues: None

Funding: Nil.

REFERENCES

[1] . Li D, He J, Ding G, Xin Y, Feng F, Ma S, et al. Advancements in NADH oxidase nanozymes: Bridging nanotechnology and biomedical applications. Adv Healthc Mater. 2024 Sep 29;e2402785.

[2] . Pijeira MSO, Viltres H, Kozempel J, Sakmár M, Vlk M, İlem-Özdemir D, et al. Radiolabeled nanomaterials for biomedical applications: radiopharmacy in the era of nanotechnology. EJNMMI Radiopharm Chem. 2022 Apr 25;7(1):8.

[3] . Sohn S-I, Priya A, Balasubramaniam B, Muthuramalingam P, Sivasankar C, Selvaraj A, et al. Biomedical applications and bioavailability of curcumin-an updated overview. Pharmaceutics. 2021 Dec 7;13(12):2102.

[4] . Malini S, Roy A, Raj K, Raju KSA, Ali IH, Mahesh B, et al. Sensing beyond senses: An overview of outstanding strides in architecting nanopolymer-enabled sensors for biomedical applications. Polymers (Basel). 2022 Feb 3;14(3):601.

[5] . Burdușel A-C, Gherasim O, Grumezescu AM, Mogoantă L, Ficai A, Andronescu E. Biomedical applications of silver nanoparticles: An up-to-date overview. Nanomaterials (Basel). 2018 Aug 31;8(9):681.

[6] . Thomas S, Gonsalves RA, Jose J, Zyoud SH, Prasad AR, Garvasis J. Plant-based synthesis, characterization approaches, applications and toxicity of silver nanoparticles: A comprehensive review. J Biotechnol. 2024 Nov 10;394:135–49.

[7] Muddapur UM, Alshehri S, Ghoneim MM, Mahnashi MH, Alshahrani MA, Khan AA, et al. Plant-based synthesis of gold nanoparticles and theranostic applications: A review. Molecules. 2022 Feb 18;27(4):1391.

[8] . Jan H, Shah M, Andleeb A, Faisal S, Khattak A, Rizwan M, et al. Plant-based synthesis of zinc oxide nanoparticles (ZnO-NPs) using aqueous leaf extract of Aquilegia pubiflora: Their antiproliferative activity against HepG2 cells inducing reactive oxygen species and other in vitro properties. Oxid Med Cell Longev. 2021 Aug 17;2021(1):4786227.

[9] . Omidtorshiz A, Benam MR, Momennezhad M, Darroudi M. Plant-based synthesis of lead oxide nanoparticles using Trigonella feonumgraecum extract and assessment of their cytotoxicity and photocatalytic activity. J Solgel Sci Technol [Internet]. 2023 Mar 27 [cited 2025 Mar 18]; Available from: https://www.semanticscholar.org/paper/45894bd2e8d8171fc422f668961a7fb54ed0562b

[10] Sabouri Z, Sabouri M, Amiri MS, Khatami M, Darroudi M. Plant-based synthesis of cerium oxide nanoparticles using Rheum turkestanicum extract and evaluation of their cytotoxicity and photocatalytic properties. Mater Technol (UK). 2022 Jul 3;37(8):555–68.

[11] Hosseinpour L, Baharara J, Zaker Bostanabad S, Darroudi M. Plant-based synthesis of selenium nanoparticles using Cordia myxa fruit extract and evaluation of their cytotoxicity effects. Inorg Chem Commun. 2022 Nov;145(110030):110030.

[12] Okaiyeto K, Hoppe H, Okoh AI. Plant-based synthesis of silver nanoparticles using aqueous leaf extract of Salvia officinalis: Characterization and its antiplasmodial activity. J Cluster Sci. 2021 Jan;32(1):101–9.

[13] Foroutan Z, Afshari AR, Sabouri Z, Mostafapour A, Far BF, Jalili-Nik M, et al. Plant-based synthesis of cerium oxide nanoparticles as a drug delivery system in improving the anticancer effects of free temozolomide in glioblastoma (U87) cells. Ceram Int. 2022 Oct;48(20):30441–50.

[14] Ahmad A, Afzaal M, Saeed F, Ali SW, Imran A, Zaidi SYR, et al. A comprehensive review of the therapeutic potential of citrus bioflavonoid hesperidin against lifestyle-related disorders. Cogent Food Agric [Internet]. 2023 Dec 31 [cited 2025 Mar 18];9(1). Available from: https://www.semanticscholar.org/paper/6bc95f530628da8e2bf8f5de7e70b03911162e5e

[15] Kumar D, Dey YN, Pramanik SD, Singh LP, Selvaraja M, Rajagopal M. Potential of hesperidin in the medicinal field. Curr Bioact Compd [Internet]. 2023 Jun [cited 2025 Mar 18];19(5). Available from: https://www.semanticscholar.org/paper/0c5d3a9b96a46ba4b0308928b5d3673abb029793

[16] Carneiro Ferreira I, Pereira da Silva V, Vilvert JC, de França Souza F, de Freitas ST, Dos Santos Lima M. Brazilian varieties of acerola (Malpighia emarginata DC.) produced under tropical semi-arid conditions: Bioactive phenolic compounds, sugars, organic acids, and antioxidant capacity. J Food Biochem. 2021 Jun 23;45(8):e13829.

[17] Kaur S, Singh V, Chopra HK, Panesar PS. Extraction and characterization of phenolic compounds from mandarin peels using conventional and green techniques: a comparative study. Discov Food [Internet]. 2024 Jul 18 [cited 2025 Mar 18];4(1). Available from: https://www.semanticscholar.org/paper/ebe1ec72b1c9f2af4fb735bea1a649bfdd4e1c69

[18] Rangsinth P, Sharika R, Pattarachotanant N, Duangjan C, Wongwan C, Sillapachaiyaporn C, et al. Potential beneficial effects and pharmacological properties of ergosterol, a common bioactive compound in edible mushrooms. Foods [Internet]. 2023 Jun 29;12(13). Available from: http://dx.doi.org/10.3390/foods12132529

[19] Singh S, Pandey S. Review on bioactive compound, alkannin/shikonin: Isolation, biosynthesis, formulations, clinical trials, patents and toxicity with phytochemicals and pharmacological properties of an endangered species, Alkanna tinctoria. Curr Bioact Compd [Internet]. 2024 Oct 30 [cited 2025 Mar 18];21. Available from: https://www.semanticscholar.org/paper/934f4ea5242c330733e81b0042775d55380e1496

[20] Karim N, Shishir MRI, Gowd V, Chen W. Hesperidin-an emerging bioactive compound against metabolic diseases and its potential biosynthesis pathway in microorganism. Food Rev Int. 2021 Jan 18;1–23.

[21] Rodrigues CV, Pintado M. Hesperidin from orange peel as a promising skincare bioactive: An overview. Int J Mol Sci. 2024 Feb 4;25(3):1890.

[22] Dathan P, Nallaswamy D, Rajeshkumar S, Joseph S, Shahin I, Tharani M. In vitro evaluation of anti-inflammatory, anti-oxidant activity of pomegranate peel extract mediated calcium sulfate nano particles. Med J Malaysia. 2025 Jan;80(Suppl 1):44–51.

[23] Tarhan T. A detailed review on the Green synthesis of selenium nanoparticles using plant extracts and their anticancer applications. ChemistrySelect [Internet]. 2024 Nov [cited 2025 Mar 18];9(41). Available from: https://www.semanticscholar.org/paper/9445b4f1cfa0013382463d915e9c9fc1bf4b7b7a

[24] Pyrzynska K. Plant extracts for production of functionalized selenium nanoparticles. Materials (Basel). 2024 Jul 29;17(15):3748.

[25] Pyrzynska K, Sentkowska A. Biosynthesis of selenium nanoparticles using plant extracts. J Nanostructure Chem. 2022 Aug;12(4):467–80.

[26] Lazcano-Ramírez HG, Garza-García JJO, Hernández-Díaz JA, León-Morales JM, Macías-Sandoval AS, García-Morales S. Antifungal activity of selenium nanoparticles obtained by plant-mediated synthesis. Antibiotics (Basel). 2023 Jan 8;12(1):115.

[27] Villagrán Z, Anaya-Esparza LM, Velázquez-Carriles CA, Silva-Jara JM, Ruvalcaba-Gómez JM, Aurora-Vigo EF, et al. Plant-based extracts as reducing, capping, and stabilizing agents for the Green synthesis of inorganic nanoparticles. Resources. 2024 May 26;13(6):70.

[28] Alam A, Jawaid T, Alsanad SM, Kamal M, Rawat P, Singh V, et al. Solubility enhancement, formulation development, and antibacterial activity of xanthan-gum-stabilized colloidal gold nanogel of hesperidin against Proteus vulgaris. Gels. 2022 Oct 14;8(10):655.

[29] Tritean N, Dimitriu L, Dima Ștefan-O, Stoica R, Trică B, Ghiurea M, et al. Cytocompatibility, antimicrobial and antioxidant activity of a mucoadhesive biopolymeric hydrogel embedding selenium nanoparticles phytosynthesized by sea buckthorn leaf extract. Pharmaceuticals (Basel) [Internet]. 2023 Dec 22;17(1). Available from: http://dx.doi.org/10.3390/ph17010023

[30] Ahmed H, Aboul-Enein A, abou-Elella F, Salem S, Aly H, Nassrallah A, et al. Nano-formulations of hesperidin and essential oil extracted from sweet orange peel: Chemical Properties and Biological Activities. Egypt J Chem. 2021 Aug 4;0(0):0–0.

[31] Lavanya, Padmini R. An overview of discrete nanoformulated flavonoids and its implication in cancer. Int J Appl Pharm. 2023 Nov 7;69–75.

[32] Jönsson A, Foerster S, Golparian D, Hamasuna R, Jacobsson S, Lindberg M, et al. In vitro activity and time-kill curve analysis of sitafloxacin against a global panel of antimicrobial-resistant and multidrug-resistant Neisseria gonorrhoeae isolates. APMIS. 2018 Jan;126(1):29–37.

[33] van Os W, Zeitlinger M. Predicting antimicrobial activity at the target site: Pharmacokinetic/pharmacodynamic indices versus time-kill approaches. Antibiotics (Basel). 2021 Dec 4;10(12):1485.

[34] Varghese RM, S AK, Shanmugam R. Antimicrobial activity of zinc oxide nanoparticles synthesized using

Ocimum tenuiflorum and Ocimum gratissimum herbal formulation against oral pathogens. Cureus. 2024 Feb;16(2):e53562.

[35] Wulandari F, Sri Sunarsih E, Musnaini K. Characterisation an d phytochemical screening of ethanolic extract Citrus reticulata peel and its anti-inflammatory activity using protein denaturation method. Pharm Educ. 2024 Jun 14;24(6):1–6.

[36] Seethalaxmi S Dr, Shubharani S Dr. In vitro anticancer and anti-inflammatory activity of green synthesized selenium nanoparticles of Baliospermum montanum leaf on hepatocellular carcinoma. INDIAN JOURNAL OF APPLIED RESEARCH. 2023 Apr 1;3–6.

[37] Suzuki R, Maruyama K, Sato S. Anti-inflammatory effects of hesperidin on human gingival fibroblasts stimulated by lipopolysaccharide of Porphyromonas gingivalis in vitro. Odontology [Internet]. 2024 Aug 20; Available from: http://dx.doi.org/10.1007/s10266-024-00988-0

[38] Jagetia10 GC. A review on the anti-inflammatory activity of hesperidin, a bioflavonoid synthesized by citrus fruits. Immunology and Inflammation Diseases Therapy. 2018 Jun 13;1(2):01–4.

[39] Choi S-S, Lee S-H, Lee K-A. A comparative study of hesperetin, hesperidin and hesperidin glucoside: Antioxidant, anti-inflammatory, and antibacterial activities in vitro. Antioxidants (Basel). 2022 Aug 20;11(8):1618.

[40] Ibrahim FM, Abdelsalam E, Mohammed RS, Ashour WES, Vilas-Boas AA, Pintado M, et al. Polyphenol-rich extracts and essential oil from Egyptian grapefruit peel as potential antioxidant, antimicrobial, and anti-inflammatory food additives. Appl Sci (Basel). 2024 Mar 26;14(7):2776.

[41] Alherz FA, El-Masry TA, Oriquat GA, Elekhnawy E, Al-Shaalan NH, Gaballa MMS, et al. Hesperidin nanoformulation: A potential strategy for reducing doxorubicin-induced renal damage via the Sirt-1/HIF1-α/VEGF/NF-κB signaling cascade. Pharmaceuticals (Basel). 2024 Aug 30;17(9):1144.

[42] Alotaibi BS, El-Masry TA, Selim H, El-Bouseary MM, El-Sheekh MM, Makhlof MEM, et al. New insights into the anticancer effects of Polycladia crinita aqueous extract and its selenium nanoformulation against the solid Ehrlich carcinoma model in mice via VEGF, notch 1, NF-κB, cyclin D1, and caspase 3 signaling pathway. Front Pharmacol. 2024 Feb 16;15:1345516.

[43] Pradhan SP, Behera A, Sahu PK. A comparative study of nanoconjugates of a synthetic and a natural drug against T2DM-induced cognitive dysfunction. J Neuroimmune Pharmacol. 2025 Feb 1;20(1):11.

[44] Najafi Z, Einafshar E, Mirzavi F, Amiri H, Jalili-Nik M, Soukhtanloo M. Protective effect of hesperidin-loaded selenium nanoparticles stabilized by chitosan on glutamate-induced toxicity in PC12 cells. J Nanopart Res [Internet]. 2023 Sep [cited 2025 Mar 18];25(9). Available from: https://www.semanticscholar.org/paper/958a551bb57d751157d1624de4aa5704c12317d4

[45] Fathima J N, Govindaraju L, Jeevanandan G, Maganur PC, Vishwanathaiah S, Assiry AA, et al. Evaluation of the cytotoxicity of a newly developed obturating material for pulpectomy in primary teeth using embryonic toxicology, brine shrimp lethality, and MTT assay: An in vitro study. Eur J Dent [Internet]. 2025 Mar 12; Available from: http://dx.doi.org/10.1055/s-0045-1802571

[46] Tharani M, Rajeshkumar S, Al-Ghanim KA, Nicoletti M, Sachivkina N, Govindarajan M. Terminalia chebula-assisted silver nanoparticles: Biological potential, synthesis, characterization, and ecotoxicity. Biomedicines [Internet]. 2023 May 18;11(5). Available from: http://dx.doi.org/10.3390/biomedicines11051472

[47] Kumar Singh R, Nallaswamy D, Rajeshkumar S, Varghese SS. Green synthesis of silver nanoparticles using neem and turmeric extract and its antimicrobial activity of plant mediated silver nanoparticles. J Oral Biol Craniofac Res. 2025 Mar;15(2):395–401.

[48] Rajeshkumar S, Tharani M, Rajeswari VD, Alharbi NS, Kadaikunnan S, Khaled JM, et al. Synthesis of greener silver nanoparticle-based chitosan nanocomposites and their potential antimicrobial activity against oral pathogens. Green Process Synth. 2021 Oct 22;10(1):658–65.

© 2024 Journal of Carcinogenesis | Published for Carcinogenesis Press by Wolters Kluwer-Medknow