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STEM-MEDIATED SYNTHESIS ON ANDROGRAPHIS PANICULATA - AN ANTI INFLAMMATORY AND EMBRYONIC TOXICOLOGY EVALUATION OF SELENIUM NANOPARTICLES

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ABSTRACT
Introduction: Traditional medicine has made use of the anti-inflammatory qualities of Andrographis paniculata to enhance patient outcomes. The negligible toxicity and biological benefits of selenium nanoparticles (SeNPs) make them interesting. This work investigates the synthesis of selenium nanoparticles utilizing extracts from Andrographis paniculata using a STEM-mediated method, and assesses the toxicity and anti-inflammatory properties of the resulting material for developing embryos. **Aim:** To synthesize selenium nanoparticles using Andrographis paniculata extracts and assess their anti-inflammatory properties and embryonic toxicity to evaluate their potential for biomedical applications. **Material and methods :** Using extracts from Andrographis paniculata, selenium ions were reduced to generate selenium nanoparticles. Using scanning transmission electron microscopy (STEM), the nanoparticles was evaluated. Using a panel of tests, comprised of the bovine serum albumin (BSA) assay for protein denaturation, the membrane stabilization assay for erythrocyte membrane protection, and the Egg Albumin Denaturation Assay is used to evaluate the anti-inflammatory and analgesic properties of substances by measuring their ability to inhibit the denaturation of egg albumin, a model protein. Zebrafish embryo models are employed for evaluating the toxicity of embryonic materials, in addition to hatching and viability rates have been recorded using graphical analysis. **Results:** The production of nanoparticles made of selenium with an identical size distribution was validated by STEM analysis. Profound anti-inflammatory effects were demonstrated by anti-inflammatory assays, which demonstrated a considerable suppression of protein denaturation in the BSA assay, increased membrane stabilization in the membrane stabilization assay, and decreased erythrocyte aggregation in the EA assay. Adverse effects were dose-dependent at higher doses, but at lower concentrations zebrafish embryo toxicity tests showed little harm. The viability and hatching rate graphs showed that selenium nanoparticles were well-tolerated at the right doses. **Conclusion:** With the help of Andrographis paniculata extracts, selenium nanoparticles were effectively synthesized, and at the right doses, they demonstrated strong anti-inflammatory efficacy and minimal embryonic damage. These findings point to the possibility of using selenium nanoparticles mediated by Andrographis paniculata in anti-inflammatory treatments and other biological contexts.

Key Words: Andrographis paniculata, selenium nanoparticles,anti-inflammatory, embryonic damage,Egg Albumin Denaturation Assay,Zebrafish embryo toxicity

INTRODUCTION

Nanotechnology is the process of creating nanoparticles with perfect characteristics and nanoscale dimensions.Nanoparticles are much more surface-area-rich than bulk materials. The subject of developing bioinspired nanostructures is one that is expanding quickly in contemporary technology.Natural or artificial materials that contain particles in their free state or as aggregates,

with 50% or more of the particles having dimensions between 1 and 100 nm in terms of size, number, distribution, or one or more exterior dimensions, are referred to as nanomaterials.

One of the most exciting new technologies that offers up a plethora of creative applications in the sciences is nanotechnology.Current research is very interested in the environmentally friendly synthesis of nanoparticles using microorganisms, algae, and plant components.Vitis vinifera, 4 Cissus quadrangularis, 5 Piper nigrum, 6 Garcinia mangostana, 7 Nitraria schoberi, 8 Abelmoschus esculentus, 9 Medicago sativa, 10 are the plants utilized to synthesize nanoparticles.(2)

In the past few decades, nanotechnology has been the greatest help to science and technology. Its exponential expansion has drastically changed the fields of materials science, biomedicine, the environment, agriculture, and industry.1–5. Discovered nanomaterials include carbon nanotubes (CNTs), metals (Cu, Se), metal oxides (cerium oxide (CeO2), magnesium oxide (MgO), fullerenes, quantum dots (CdSe and CdTe), dendrimer-bound materials, and polymeric materials. These nanomaterials are widely used in a variety of biomedical applications.Six Furthermore, because of their immense potential for the transport of medications, proteins, genes, and siRNA, metal nanoparticles including Selenium(Ag), gold (Au), cerium (Ce), iron (Fe), and selenium (Se) have established a unique place in the field of nanotechnology.(3) Jons Jacob Berzelius made the earliest discovery of selenium (Se), a chemical element vital to human health.Zebrafish have become well-recognized animal models for studying tissue regeneration due to their remarkable similarity to humans. The regenerative processes observed in structures such as the caudal fin, retina, and spinal cord are highly organized in these animals, providing an excellent platform for advancing research in regenerative medicine.(4) (5).(5,6).The scientific community is interested in using Se nanoparticles (SeNPs) as therapeutic and theranostic agents since they are more biocompatible and have less toxicity than organic or inorganic Se molecules.(7). Selenium, as a key minor component, is known to enhance or restore the activity of the seleno-catalyst and glutathione peroxidase in the protection of free radical damage to cells and tissues in vivo.(8).It is frequently used in dietary supplements and may also be a components in fertilizers.(8,9).
Due to its pleiotropic impact and great therapeutic potential, the trace element selenium (Se) is gaining a lot of attention in the fields of nanomedicine and biotherapy. The World Health Organization (WHO) states that adult men and women consume 70 μg and 55 μg of selenium per day, respectively.(10). Due to its remarkable sensitivity to even very low levels of environmental contaminants, the zebrafish is an excellent model animal for assessing the toxicity of NP [von Westernhagen, 1988]. Moreover, embryos are easily obtained in great quantities, are tiny and translucent, and develop very quickly.(10,11).Non-toxic nanoparticles are specifically required for biomedical applications. However, since nanoparticles used in manufacturing and other applications may expose people to the environment, toxicity is also a crucial factor to take into account.(12).(13)(13,14).There are several platforms for evaluating toxicity, from in vitro cell culture experiments to simple model species like sea urchins and daphnia to sophisticated higher vertebrate models like mice and primates . For investigations on cell-level toxicity and genotoxicity, cell lines and simple creatures are helpful, but higher vertebrates are necessary to identify intricate physiological relationships. But compared to primate models, which have comparable but more severe problems, rodent models are more expensive, have embryos that develop slowly and are difficult to access, need a lot of material to verify because of their size, and raise ethical questions about their use.Silver nanoparticles have potential for antimicrobial activity. Mini-implants placed as

temporary anchorage devices in orthodontics often fail due to inflammation and plaque. Silver nanoparticle-coated mini-implants would reduce the risk of mini-implant failure as it would have antimicrobial potential and eliminate this cause for failure of mini-implants.(15)Nano-silver (AgNPs) possesses important biological properties that make it valuable in consumer products, food technology, and a range of medical applications, including wound care, implantable devices, diagnostics, drug delivery, and imaging. As a result, its use in medicine is rapidly increasing. Nevertheless, the impact of AgNPs on zebrafish is still not well understood.(1) (16) .
There is good evidence that Se may have an impact on the course and outcome of a number of etiologically inflammatory diseases. Although the data come almost exclusively from in vitro studies, there is strong indication that viral, bacterial, or stress induced inflammation may be variably influenced by Se availability. Decreased serum Se levels have been observed in acute and chronic inflammatory states with high CRP values(17).Significant inflammatory response syndrome (SIRS), characterized by elevated reactive oxygen species (ROS) production by activated macrophages, induction of oxidative damage, and tissue destruction, has also been linked to low Se levels.(17,18).Se's impact on immune cells, particularly on the signal transduction pathways of macrophages, is associated with its anti-inflammatory properties. According to a recent study, selenium supplementation significantly reduces the expression of the primary proinflammatory genes TNF-a and cyclooxygenase-2 (COX-2) caused by the bacterial endotoxin lipopolysaccharide (LPS) by blocking the MAP kinase pathways.

Antioxidants, which neutralize free radicals, may assist to stop some of the damage that they cause. Certain foods include antioxidants.These consist of copper, zinc, and selenium minerals as well as antioxidants and the vitamins A, C, and E. It was previously believed that other dietary food molecules, or phytochemicals, which are present in plants, had stronger antioxidant qualities than vitamins or minerals.We refer to phytochemicals (such the lycopenes in tomatoes and the anthocyanins in cranberries) as non-nutrient antioxidants. A substance that stops other substances from oxidizing is known as an antioxidant.By preventing the harmful effects of free radicals, which are created during cell metabolism from natural byproducts, they safeguard vital cell components.The annual herb Andrographis (A.) paniculata is an erect member of the Acanthaceae family. This medicinal herb, which has a strong bitter taste, is used to treat digestive problems, liver illnesses, colic discomfort, and upper respiratory infections.(19)

The antibacterial properties are not completely known, and more research is necessary to determine whether they could be harmful to human tissues.
With the use of ligands or antibodies to modify the surface, targeted NP delivery to the infection site may also be accomplished. This could increase treatment efficacy and lessen adverse medication reactions. Compounds that function as ligands and boost Sn NPs' uptake into bacterial cells, such as polyethylene imines, chitosan, and glucosamine, also increase the efficacy of the particle suspensions.(20)

Ag, Au, Ce, Fe, Se, Si, Ti, and Zn metal nanoparticles hold a unique position in the field of nanotechnology since they currently being developed a special chance to function as therapeutic agents.One of these that has been explored the most is selenium nanoparticles.Selenium nanoparticles, or SeNPs, are among these nanoparticles that have been investigated the most and have shown to have important potential in the medical area (3). When compared to other Se species, SeNPs

have appealing anticancer efficacy and less safety concerns. SeNPs have been applied to numerous health-related issues including as drug-induced toxicities, inflammatory disorders, cancer, diabetes, and liver fibrosis.(21)

In addition to enhancing immunity, selenium also shields tissues from oxidative stress and supports growth, development, reproduction, and modulation. The range of selenium's bioactivity to toxicity is rather narrow. In comparison to physicochemical approaches, biological synthesis of selenium exhibits greater biological activity, bioavailability, and reduced toxicity.Thus far, these nanoparticles' exceptional antibacterial efficacy against harmful bacteria, fungus, and yeasts has been demonstrated.(22)

Asia is home to the well-known medicinal herb Andrographis paniculata, which is a member of the Acanthaceae family. Its antimicrobial, antiviral, antifungal, and antiparasitic properties are well known in the medical community. Andrographolide (AG), the active ingredient in A. paniculata, is known to suppress the virulence factor of bacteria and is suspected to be a quorum-sensing inhibitor.It controls the phenotypic polarization of macrophages, which in turn controls host immunity and generates antibodies specific to Ag.The antimicrobial efficacy of A. paniculata against Staphylococcus aureus and Candida albicans was tested by Dedhia et al. using it as a root canal irrigant. The results were compared with sodium hypochlorite and showed a significantly higher zone of inhibition against C. albicans and a comparable zone of inhibition against S.aureus.(23) (14) .Andrographis paniculata is widely cultivated and its importance as a medicinal plant is growing up with stronger reports in support of its multifarious therapeutic uses. Taking great concern of the useful benefits of the plant, it can be advocated as a safe, highly important medicinal plant for mankind. This herb contains a large number of chemical constituents, mainly lactones, diterpenoids, diterpene glycosides, flavonoids and flavonoid glycosides. It has multiple pharmacological properties such as antibacterial, hepatoprotective activity, anti-cancer, antitumor, hypoglycemic, immunomodulatory and hypotensive activities.
(24).

Material and method

Wash the andrographis paniculata leaves thoroughly with clean water to remove any dirt, pat them dry with a clean cloth. Grind the leaves into a coarse powder using a grinder. In a container, add the powdered leaves to distilled water. Filter the extract using filter paper or cheesecloth to remove the plant material. Collect the filtrate, which is the aqueous extract of andrographis paniculata.

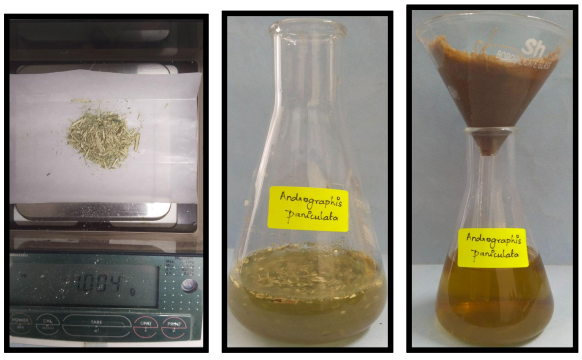


Figure 1: Preparation of plant extract

Figure 2: dried andrographis paniculata in distilled water
Figure 3: filtered extract of andrographis paniculata

Prepare a solution of Sodium selenate (AgNO3) in distilled water. Slowly add the Sodium selenate solution solution to the prepared Andrographis paniculata extract under constant stirring. Monitor the reaction by observing the color change of the solution. The formation of silver nanoparticles often results in a color change, typically yellow or brown(figure 6).

Figure 4: preparation of selenium nanoparticles
Figure 5: sodium selenate solution in a beaker
Figure 6: andrographis paniculata with sodium selenate solution

Anti-inflammatory activity:
Bovine serum albumin denaturation assay
The green synthesized Seleniumnanoparticles were tested for their anti-inflammatory activity using two assays Bovine serum albumin denaturation assay. 0.45 mL of bovine serum albumin was mixed with 0.05 mL of different concentrations (10, 20, 30 ,40, 50 µg/mL) of A. paniculata mediated Seleniumnanoparticles. The pH was adjusted to 6.3. Then it was kept at room temperature for 10 minutes followed by incubation in a water bath at 55°C in a water bath for 30 min. Diclofenac sodium was used as the standard group while dimethyl sulphoxide was used as control. Then, the samples were measured spectrophotometrically at 660nm.

The percentage of protein denaturation was determined utilizing the following equation,

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample} \times 100}{\text{Absorbance of control}}$$

Egg Albumin denaturation assay
To perform the egg albumin denaturation assay, 0.2 mL of fresh egg albumin was mixed with 2.8 mL of 1X phosphate buffer. Different concentrations (10, 20, 30 ,40, 50 µg/mL) of A. paniculata-mediated Seleniumnanoparticles were added to the reaction mixture. The pH was adjusted to 6.3. Then it was kept at room temperature for 10 minutes followed by incubation in a water bath at 55°C in a water bath for 30 min. Diclofenac sodium was used as the standard group while dimethyl sulphoxide was used as control. Then, the samples were measured spectrophotometrically at 660nm.

The percentage of protein denaturation was determined utilizing the following equation,

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample} \times 100}{\text{Absorbance of control}}$$

Membrane stabilization assay:

The *in vitro* membrane stabilization assay is a widely used technique for evaluating the membrane stabilizing properties of natural and synthetic compounds. This assay measures the ability of a compound to stabilize the cell membrane by preventing its disruption and subsequent release of intracellular contents. The materials include Human red blood cells (RBCs), Phosphate-buffered saline (PBS), Tris-HCl buffer (50 mM, pH 7.4), Different concentrations of selenium nanoparticles (10, 20, 30 ,40, 50 µg/mL), Centrifuge tube, UV-Vis spectrophotometer.

Preparation of RBC suspension:

Collect fresh human blood in a sterile tube containing anticoagulant. Centrifuge the blood at 3000 RPM for 10 minutes at room temperature to separate the RBCs from other blood components. Remove the supernatant and wash the RBCs three times with PBS. Resuspendthe RBCs in the Tris-HCl buffer to obtain a 10% (v/v) RBC suspension.

Assay procedure:

Pipette 1 mL of the RBC suspension into each centrifuge tube. Then different concentrations of Selenium Nanoparticles (10, 20, 30 ,40, 50 µg/mL) were added to each tube. Mix gently and incubate the tubes at 37°C for 30 minutes. Centrifuge the tubes at 2500 RPM for 5 minutes at room temperature to pellet the RBCs. Measure the absorbance of the supernatant at 560 nm using a UV-Vis spectrophotometer.

Calculate the percentage inhibition of haemolysis using the following formula:

% inhibition = [(OD control – OD sample) / OD control] x 100

Where OD control is the absorbance of the RBC suspension without the test compound(s) and OD sample is the absorbance of the RBC suspension with the test.

Zebrafish embryonic toxicology evaluation of Seleniumnanoparticles

Fish maintenance and AgNPs exposure

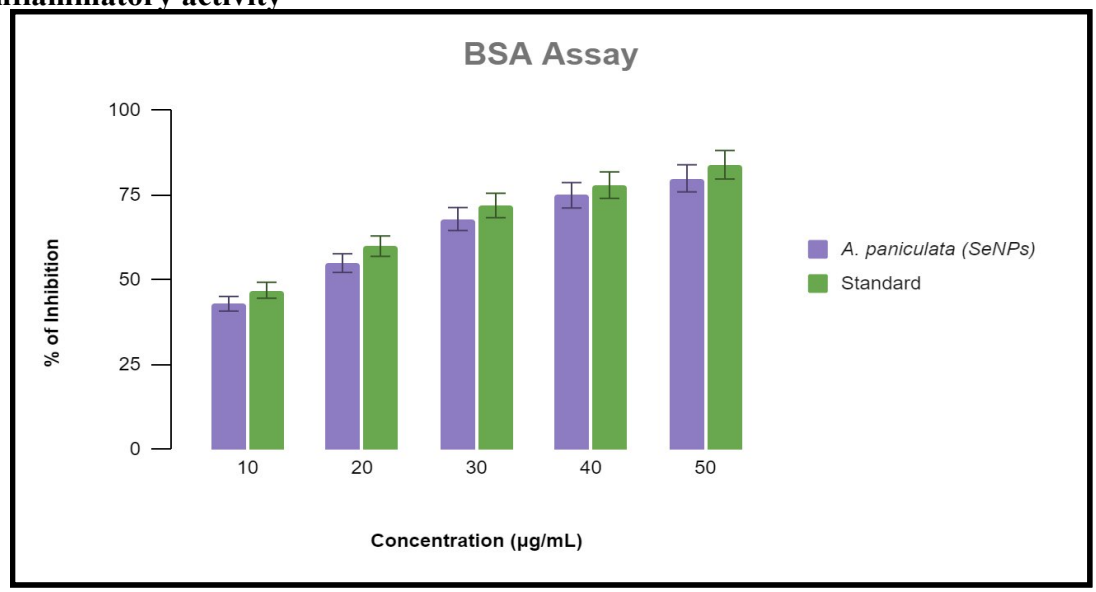
Wild-type zebrafish (*Danio rerio*) were acquired from local Indian vendors and were housed in individual tanks under controlled conditions of temperature ($28^0 \pm 2^0$C), light/dark cycle (14:10 h), and pH (6.8–8.5). The fishes were fed with commercially available dry blood worms or optimum food twice daily. Zebrafish embryos were obtained by crossing one female and three males per breeding tank, and viable eggs were collected and rinsed at least three times with freshly prepared E3 medium without methylene blue. The study involved the placement of fertilized eggs in culture plates of varying well sizes (6, 12, and 24 wells) with 20 embryos per 2 mL solution per well. The experimental treatment and control groups were replicated three times. To prepare the experimental treatment, a stock suspension of TCF-SeNPs with five different concentrations was freshly made and added directly to the E3 medium. The solution was sonicated for 15 minutes to disperse the nanoparticles while maintaining a pH range of 7.2-7.3. Healthy fertilized embryos were exposed to different concentrations of SeNPs ranging from (5, 10, 20, 40, and 80 µg/mL) for 24 to 96 hours post fertilization. The SeNPs were added to the E3 medium where the embryos were incubated. Control groups were also included in the experiment. Dead embryos were removed from the nanoparticles exposed groups every 12 hours. All experimental plates were wrapped in foil to exclude light and maintained at 28°C.

Zebrafish embryo evaluation

Throughout the exposure period following fertilization, the developmental stages of Zebrafish embryos were monitored using a stereo microscope. The embryos were subjected to various concentrations of selenium nanoparticles (5, 10, 20, 40, and 80 µg/mL) for 24-78 hpf. Embryonic mortality and hatching rates were assessed at 24-hour intervals. The study endpoints included embryo/hatchling mortality, hatching rate, and the identification and documentation of any malformations among the embryos and larvae in both control and treatment groups. Photographs of malformed embryos were captured using a COSLAB - Model: HL-10A light microscope and the percentage of abnormal embryos was recorded every 24 hours.

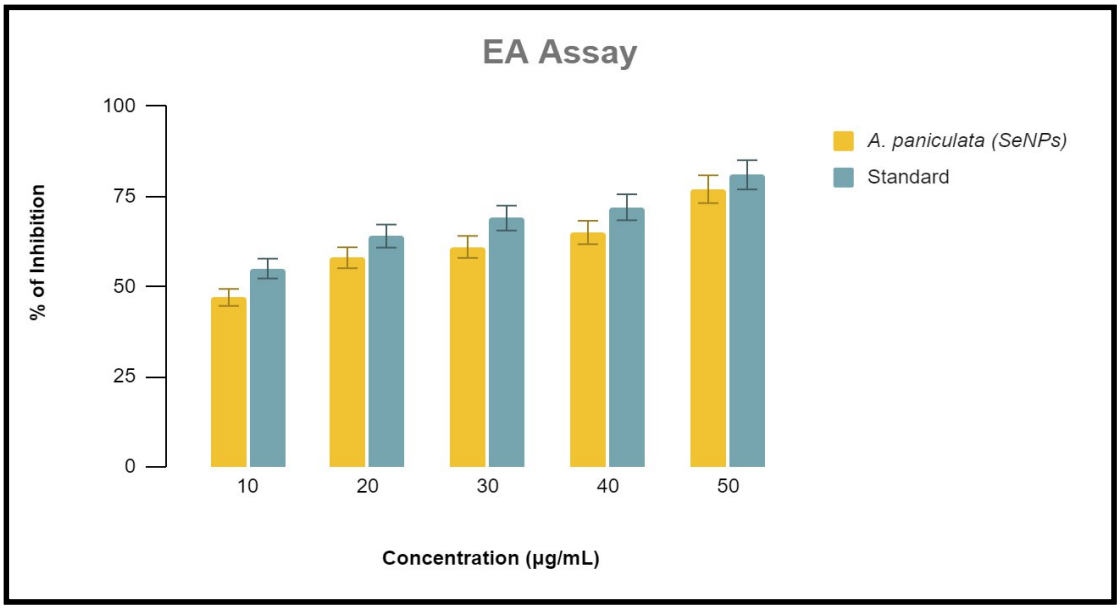
RESULT

Anti-inflammatory activity



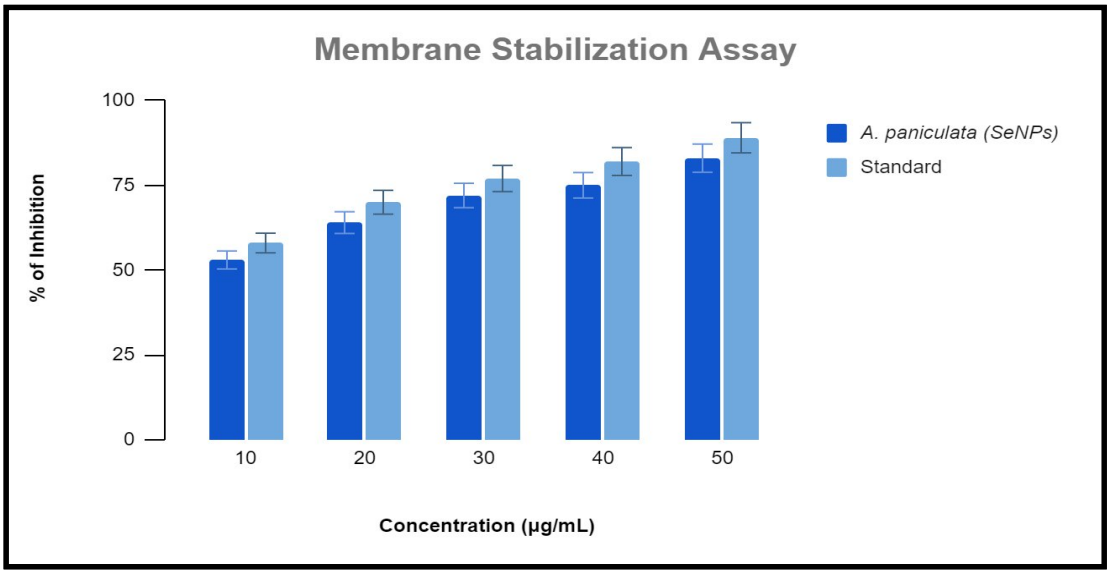
Graph 1
BSA Assay

BSA Assay showing the % inhibition ofAndrographis paniculata selenium nanoparticles(SeNPs) compared to the standard across various concentrations (10-50 µg/mL



GRAPH 2
EA Assay

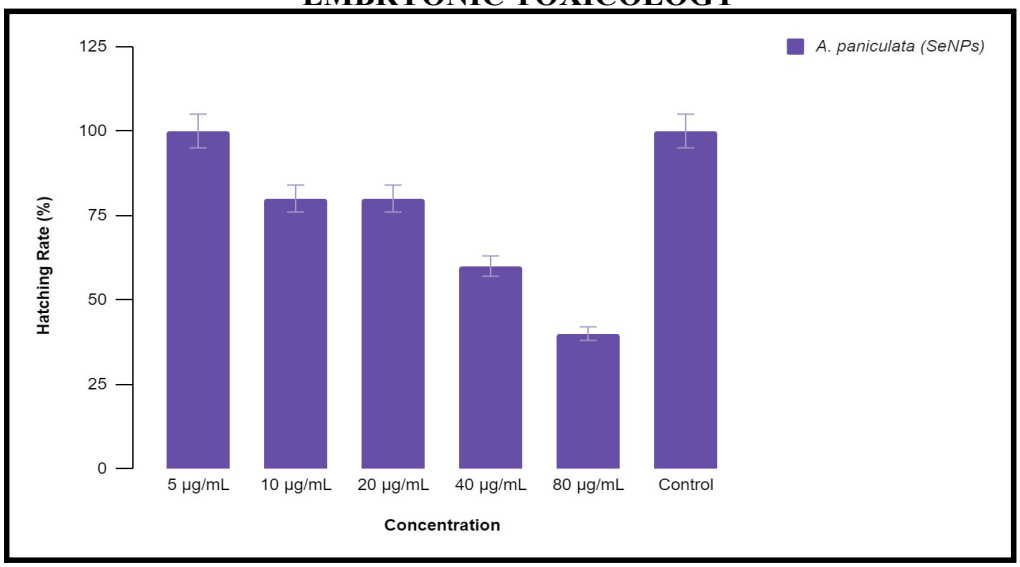
EA Assay depicting the % inhibition of Andrographis paniculata SeNPs versus the standard at different concentrations (10-50µg/mL).



GRAPH 3
Membrane Stabilization Assay

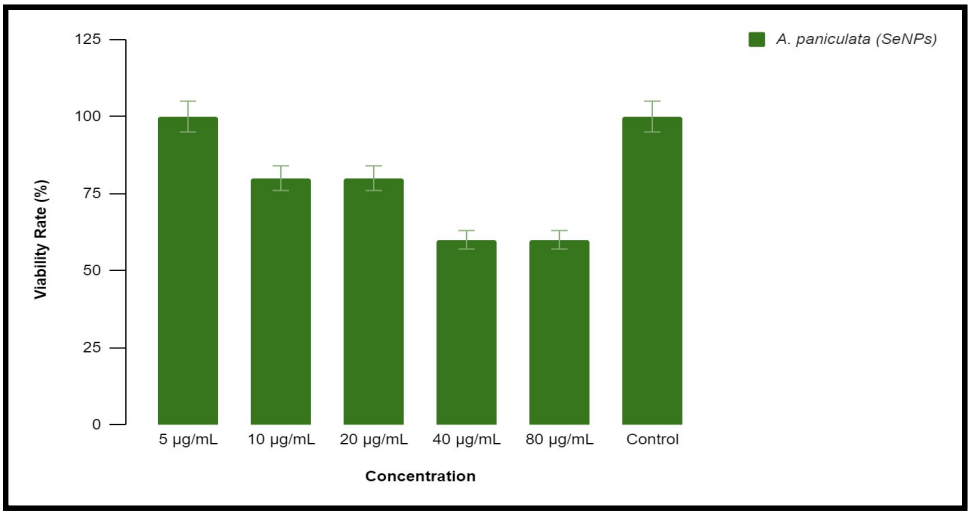
Membrane Stabilization Assay illustrating the % inhibition of Andrographis paniculata SeNPs compared to the standard at concentrations ranging from 10 to 50 µg/mL.

EMBRYONIC TOXICOLOGY



GRAPH 4
HATCHING RATE

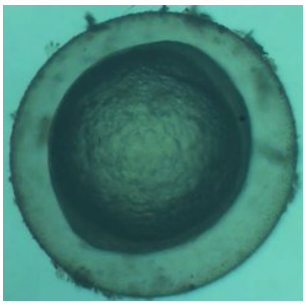
Hatching rate of embryos exposed to varying concentrations of Andrographis paniculata SeNPs (5-80 µg/mL) compared to the control.



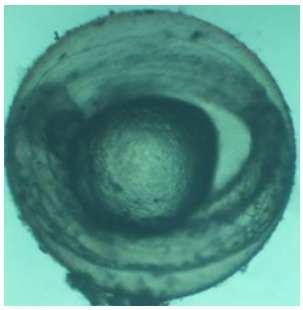
GRAPH 5
VIABILITY RATE

graph shows the effect of different A. paniculata (SeNPs) concentrations (x-axis) on cell viability rate (y-axis).

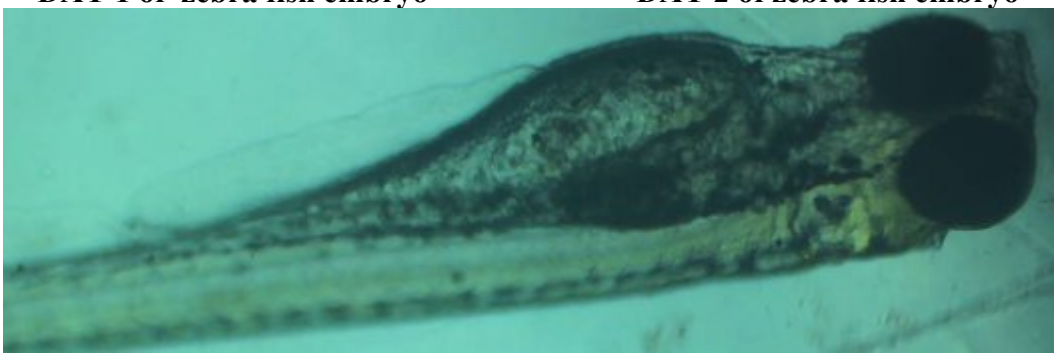
STAGES OF ZEBRA FISH



DAY 1 of zebra fish embryo



DAY 2 of zebra fish embryo



DAY 3 of zebra fish embryo

Discussion

The graph 1 compares the anti-inflammatory activity of Andrographis paniculata selenium nanoparticles (SeNPs) and a standard substance, measured by a BSA assay. The x-axis shows concentrations (10-50 µg/mL), while the y-axis indicates the percentage of inhibition of protein denaturation. Purple bars represent *A. paniculata* SeNPs, and green bars represent the standard. Both show dose-dependent increases in inhibition, with error bars indicating measurement variability.

The graph 2, measures the ability to prevent hypotonicity-induced hemolysis, indicating anti-inflammatory activity. The x-axis shows concentrations (10-50 µg/mL), while the y-axis indicates the percentage of inhibition. Yellow bars represent *A. paniculata* SeNPs, and teal bars represent the standard. Both show dose-dependent increases in hemolysis inhibition, with error bars indicating measurement variability.

The graph 3,evaluates the ability to stabilize the lysosomal membrane, preventing the release of inflammatory mediators. The x-axis shows concentrations (µg/mL), while the y-axis indicates the percentage of inhibition. Dark blue bars represent *A. paniculata* SeNPs, and light blue bars represent the standard. Both show dose-dependent increases in membrane stabilization, with error bars indicating measurement variability.

The graph 4 , shows embryonic toxicity of selenium nanoparticles (SeNPs) from *Ageratum paniculatum* at different concentrations, with the x-axis indicating SeNP concentration and the y-axis showing hatching rate. Two panels display viability and hatching rates. Both panels show a decrease in hatching rate as SeNP concentration increases. At 5 µg/mL, hatching rates are about 100%

for both control and *A. paniculata* groups. As concentration increases, the *A. paniculata* group's hatching rate decreases more rapidly. At 80 µg/mL, the control group's hatching rate is about 75%, while the *A. paniculata* group's is about 10%.
The graph 5, x-axis of the graph shows SeNP concentration, and the y-axis shows embryo viability. As SeNP concentration increases, embryo viability decreases. At 5 µg/mL, viability is about 100% for both control and *A. paniculata* groups. As concentration increases, the *A. paniculata* group's viability decreases more rapidly. At 80 µg/mL, the control group's viability is about 75%, while the *A. paniculata* group's is about 25%.

In the previous study , A brand-new, easy-to-follow procedure is suggested for employing BSA to create selenium nanoparticles with a diameter of 500 nm. This technique yields very stable and cost-effective selenium nanoparticles. The selenium nanoparticles may be important in future applications in medicine because of their biological characteristics. Utilizing zebrafish embryos, toxicity tests were conducted in order to determine the therapeutic concentrations. The findings show that 5–10 µg/ml of SeNPs may be an economically viable option for cardiovascular illnesses.A novel one-pot green synthesis of selenium nanoparticles and ….

Also in another study , Anti-inflammatory Properties of Selenium Nanoparticles (SeNPs)
The anti-inflammatory potential of selenium nanoparticles (SeNPs) synthesized using Andrographis paniculata (A. paniculata) is supported by various studies. Selenium is a crucial trace element known for its antioxidant properties, which contribute to its anti-inflammatory effects. Studies have shown that SeNPs can inhibit the production of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 by modulating signaling pathways such as NF-κB and MAPK. The bioactive compounds in A. paniculata, particularly andrographolide, have been reported to enhance the anti-inflammatory effects of SeNPs. For instance, a study by demonstrated that andrographolide-loaded SeNPs exhibited superior anti-inflammatory activity compared to SeNPs alone, indicating a synergistic effect between the plant extract and the nanoparticles.(25).(26)

Embryonic Toxicology of SeNPs
While the anti-inflammatory benefits of SeNPs are promising, their embryonic toxicology is a critical aspect that requires thorough investigation. Previous research has indicated that nanoparticles, depending on their size, dose, and surface properties, can cross biological barriers and interact with embryonic tissues, potentially causing developmental toxicity. Studies on the embryotoxicity of SeNPs have shown mixed results.It was found that low concentrations of SeNPs did not induce significant toxic effects on zebrafish embryos, whereas higher concentrations led to developmental abnormalities and oxidative stress. These findings underscore the importance of careful dose optimization in the biomedical application of SeNPs.(27)

Combined Effects of A. paniculata and Senps.
The combination of A. paniculata and SeNPs presents a novel approach for enhancing therapeutic efficacy while potentially mitigating toxic effects. Andrographolide, the main active component of A. paniculata, has been documented for its anti-inflammatory and antioxidant properties, which may synergize with the characteristics of SeNPs (Singha et al., 2017). Moreover, plant-mediated synthesis of nanoparticles, including SeNPs, has been reported to yield biocompatible and less toxic formulations compared to chemically synthesized counterparts (Mittal et al., 2018). This green

synthesis approach leverages the bio-reducing and stabilizing agents present in the plant extract, resulting in nanoparticles with enhanced stability and reduced cytotoxicity (Ahmad et al., 2020).
In conclusion, the STEM-mediated synthesis of SeNPs using A. paniculata offers a promising strategy for developing anti-inflammatory agents with potentially reduced embryonic toxicity. However, further in-depth studies are required to elucidate the exact mechanisms underlying the interactions between SeNPs and biological systems, and to optimize the formulation for safe and effective therapeutic use.

Conclusion

The biogenic synthesis of selenium nanoparticles using Andrographis paniculata extract presents a sustainable and eco-friendly alternative to traditional chemical methods. This approach utilizes the plant's bioactive compounds to reduce Sodium selenate.($AgNO_3$) in an aqueous solution, forming selenium nanoparticles indicated by a color change. This method minimizes the use of toxic chemicals, reduces environmental pollution, and incorporates the therapeutic properties of Andrographis paniculata, particularly its anti-inflammatory effects. The resulting nanoparticles hold promise for various biomedical applications, including drug delivery and treatment of inflammatory diseases.Future research should aim to optimize synthesis parameters for consistent production, understand the biochemical mechanisms involved, and conduct comprehensive toxicological evaluations to ensure safety and efficacy. This green synthesis method underscores the potential of combining sustainable practices with advanced nanotechnology for innovative and environmentally responsible solutions.

CONFLICT OF INTEREST

There was no conflict of interest

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