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In Vitro Cytotoxic Analysis of Solanum Xanthocarpum induced silver nanoparticles on Brine Shrimp Artemia

Running title:Cytotoxicity analysis of Solanum Xanthocarpum induced silver nanoparticles

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Abstract

Introduction

In the branch of nanotechnology, the synthesis of nanoparticles and their development play a significant role because of their wide applications. They are used as antimicrobial agents and prevent the development of multidrug resistant bacteria.All parts of Solanum xanthocarpum plant, including the stem, flowers, fruits and roots have proved to have medicinal properties. This study aimed to evaluate cytotoxic activity of silver nanoparticles synthesised using Solanum Xanthocarpum extract on brine shrimp artemia.

Materials and Methods

The cytotoxicity of silver nanoparticles reinforced solanum xanthocarpum fruit extract was assessed using Brine shrimp assay. 6 well ELISA plates were taken and to each plate 6-8 ml of saltwater was added; followed by adding 10 nauplii to each well. Silver nanoparticles reinforced with solanum xanthocarpum fruit extract was added to each well at different concentrations (5 µL, 10 µL, 20 µL, 30 µL, 50 µL) and was then incubated for 24 h. After 24 h, the total number of live and dead nauplii was counted and the mortality rate was checked.

Results

At 5 µL concentration,there was a death of 10% of nauplii, at 10 and 20 µL there was

a death of 20% and 30% of nauplii, at 40 µL there was a death of 30% of nauplii and at 80 µL there was a death of 40% of nauplii. It was seen that as the concentration increased the cytotoxicity of the nanoparticles increased.

Conclusion

The use of fruits for the synthesis of silver nanoparticles has many advantages such as, ease with which the process can be scaled up, economic viability and to obtain smaller particle size.The use of lower concentration had lower cytotoxic effects.

Keywords

Solanum Xanthocarpum;cytotoxicity;Brine shrimp artemia;Silver nanoparticles

Introduction

Nanotechnology is the development and manufacture of nanometer size range of less than 100 nm and their application.(Schmid and Riediker 2008)The biodistribution, bioactivity and interactions of nanoparticles with fluids, cells, and tissues needs to be considered.Silver nanoparticles are well known for their antimicrobial properties.(Yoon et al. 2007; Tian et al. 2007) The stability and mobility of AgNPs in the aquatic environment depends on AgNPs agglomeration, aggregation, dispersion, sedimentation and dissolution. This depends on the particle physicochemical properties and parameters such as pH, temperature, ionic strength,presence of ligands ,natural organic matter.(Handy et al. 2008; Navarro et al. 2008; Fabrega et al. 2011)

Artemia also known as brine shrimp is zooplankton that is used to feed the larval fishes.(Sorgeloos and Others 1980)The use of Artemia in toxicology helps in understanding practical considerations of laboratory culture,attainment of cyst, ecological relevance, systematic use, and practical conditions of maintenance.(Blaise 1998)

Previously our team has a rich experience in working on various research projects across multiple disciplines (L. Govindaraju and Gurunathan 2017; A. Christabel et al. 2016; Soh and Narayanan 2013; Mehta et al. 2019; Ezhilarasan, Apoorva, and Ashok Vardhan 2019; Campeau et al. 2014; Kumar and S 2016; S. L. Christabel 2015; Kumar and Rahman 2017; Sridharan, Ramani, and Patankar 2017; Ramesh et al. 2016; Thamaraiselvan et al. 2015; Thangaraj et al. 2016; Ponnulakshmi et al. 2019; “Fluoride, Fluoridated Toothpaste Efficacy and Its Safety in Children - Review” 2018) Now the growing trend in this area motivated us to pursue this project.

The objective of this study was to evaluate the in vitro cytotoxic effect of solanum xanthocarpum mediated silver nanoparticles on Brine shrimp Artemia.

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Materials and Methods

Collection of Plant Materials

Fruit of Solanum xanthocarpum was collected from Tamilnadu, India. The collected plant materials were brought to the laboratory for plant extraction and for synthesis of silver nanoparticles.

Processing of Plant Materials

The collected Solanum xanthocarpum's fruit was separated from the plant, it was washed thoroughly with tap water and then with sterile water for 2-3 times. Then it was cut into small pieces for quick drying. Cleaned fruits were shade dried for 10-15 days. The dried plant materials were crushed into fine powder with the help of an electric grinder. Finally the fine powder was stored in an airtight container at room temperature.

Preparation of Aqueous Fruit Extract of S. Xanthocarpum

25 grams of dried fruit powder was mixed with 250 ml of water. The solution was stirred for proper mixing. Then the solution was placed in a rotating shaker (100rpm) at room temperature for 48 hours (2 days). After incubation, the extract was filtered in Whatman No: 1 filter paper. Finally the filtrate was allowed to air dry at room temperature and dried powder was stored at 4 degree C until use.

Synthesis of Silver Nanoparticles from Fruit Extract

25 ml of plant filtrate was added into 225 ml of aqueous solution of 1mM silver nitrate (AgNO) for reduction of silver nitrate into Ag+ ions and kept at room temperature for 24 hours in a rotating shaker at 28degree C. The solution was kept in the dark to avoid other biological changes. In this process silver nanoparticles were produced through reduction of silver ions to metallic silver. Silver nanoparticles were determined by the change in colour of the reaction mixture.

Extract Preparation

The collected S. xanthocarpum plant material was made into small pieces, shade-dried and coarsely powdered using a pulverizer. The coarse powders were subjected to extraction with ethanol using Soxhlet apparatus. The extracts were collected and evaporated at atmospheric pressure and the last trace of the solvents was removed.

Cytotoxic Effect

The cytotoxicity of silver nanoparticles reinforced solanum xanthocarpum fruit extract was assessed using Brine shrimp assay. 6 well ELISA plates were taken and to each plate 6-8 ml of saltwater was added; followed by adding 10 nauplii to each well. Silver nanoparticles reinforced with solanum xanthocarpum fruit extract was added to each well at different concentrations (5 µL, 10 µL, 20 µL, 30 µL, 50 µL) and was then incubated for 24 h. After 24 h, the total number of live and dead nauplii was counted and the mortality rate was checked.

Number of dead nauplii

% death = Number of dead nauplii/Number of dead nauplii − number of live nauplii × 100

Results

Analysis of silver nanoparticles in plant extract

Reduction of silver ions to silver nanoparticles were analysed by observing the colour change and UV-Vis spectroscopy.(Figure 1)

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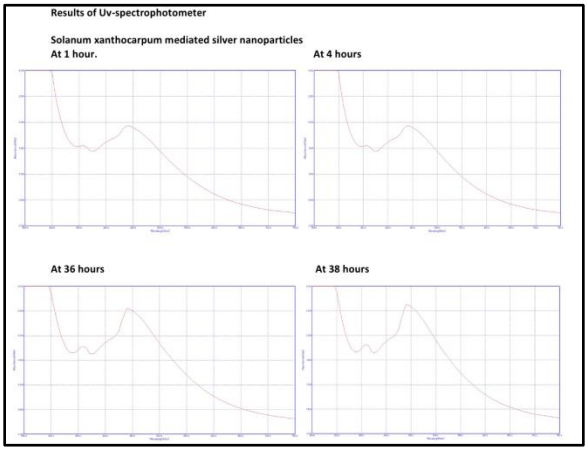
Figure 1 :Results of Uv spectrophotometer



Colour change

The synthesized silver nanoparticles were confirmed by naked observation. Production of silver nanoparticles takes place by the reduction of silver ions during exposure to the plant followed by color change. Within 2 hours the silver ions gets reduced and it exhibits colourless to dark reddish brown colour.This colour change is due to the Surface Plasmon Resonance (SPR) phenomenon.(Figure 2)

Figure 2 : Colour change observed before and after addition of silver nanoparticles

UV-VIS Spectra Analysis

Wavelength between 400-450nm the formation of silver nanoparticles reach the peak maximum.The specific characteristic peak for silver nanoparticles was due to the SPR.The UV-Visible spectrum shows the formation of silver nanoparticles of aqueous fruit extract as the peak maxima in 440 nm.This is characteristic to silver nanoparticles and the broadening of peak indicated that the particles were polydispersed.(Figure 3)

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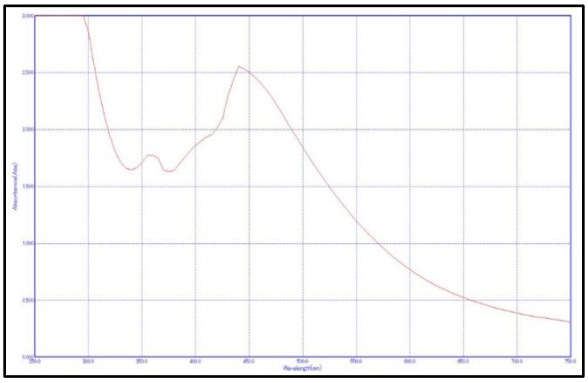
Figure 3:UV-vis spectrograph of Ag nanoparticle synthesized from Solanum xanthocarpum aqueous fruit extract



At 5 µL concentration,there was a death of 10% of nauplii, at 10 and 20 µL there was

a death of 20% and 30% of nauplii, at 40 µL there was a death of 30% of nauplii and at 80 µL there was a death of 40% of nauplii. It was seen that as the concentration increased the cytotoxicity of the nanoparticles increased.(Figure 4)

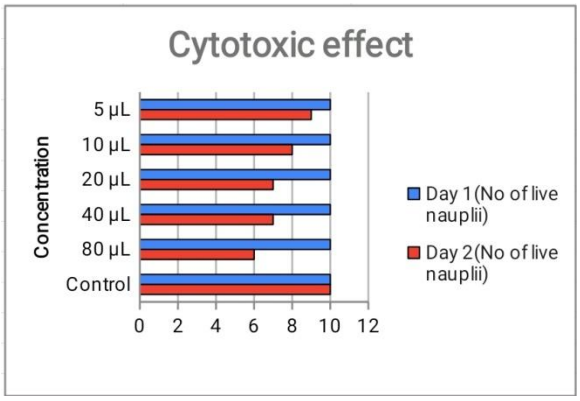


Figure 4: Depicts the cytotoxicity of Solanum Xanthocarpum induced silver nanoparticles on the nauplii

Discussion

Currently there has been a rapid evolution in nanoparticle synthesis.(Andersson, Pedersen, and Palmqvist 2005)Physio-chemical methods were involved in nanoparticle synthesis earlier. Time required for synthesizing large quantities of nanoparticles using conventional physical and chemical methods is lesser but toxic chemicals are required as capping agents to maintain stability, thereby leading to toxicity in the environment.Hence green nanotechnology using plants is emerging as an eco-friendly alternative.Plant extract mediated biosynthesis of nanoparticles is also cost-effective and proved to be more advantageous.(Chandran et al. 2006; Chen, Lin, and Ma 2003; Ingle et al. 2008)

In the present study, Ag nanoparticles from aqueous extract of fruits of Solanum xanthocarpum were studied.Ankanna et al (Ankanna et al. 2010) say by the aqueous extract it gives colour change from colourless to dark yellowish brown in colour. In the present study, the extract changed its colour from colourless to dark reddish brown.The formation of Ag nanoparticles was confirmed by UV-Vis spectral analysis. According to a study done by Govindaraju et al.,(K. Govindaraju et al. 2010) had explained the same family Solanaceae and it was found that the plant Solanum torvum got a maximum peak at around 434 nm.Our institution is passionate about high quality evidence based research and has excelled in various fields (Jayaseelan Vijayashree Priyadharsini 2019; Pc, Marimuthu, and Devadoss 2018; Ramesh et al. 2018; Ramadurai et al. 2019; Sridharan et al. 2019; Ezhilarasan,

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Apoorva, and Ashok Vardhan 2019; Mathew et al. 2020; Samuel 2021; R et al. 2020; Chandrasekar et al. 2020; J. Vijayashree Priyadharsini, Smiline Girija, and Paramasivam 2018)

This study was done to evaluate the cytotoxicity activity of silver nanoparticles reinforced with Solanum Xanthocarpum fruit extract.As the concentration increased the cytotoxicity activity of the nanoparticles increased. The highest percentage of death of nauplii was at 80ul concentration of Silver nanoparticles reinforced with Solanum Xanthocarpum fruit extract.

Conclusion

Herbal medicine is gaining growing interest because of its cost effective and echo friendliness.The reduction of the metal ions through plant extracts leading to the formation of silver nanoparticles has been explained before but the capability of parts of the plant such as fruits as a capping and reducing agent has not been explained in previous studies. In the present study, we found that fruits were a good source for the synthesis of silver nanoparticles.This study evaluated the cytotoxicity activity of silver nanoparticles obtained from Solanum Xanthocarpum fruit extract on brine shrimp larvae. As the concentration increased the cytotoxicity activity also increased. Hence lower concentrations have proved to have lesser cytotoxic effect and can be used in theraupatics.

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