CYTOTOXIC ACTIVITY OF SILVER NANOPARTICLES SYNTHESIZED USING *F.* BENGHALENSIS

ABSTRACT:
Aim: To determine the cytotoxic activity of silver nanoparticles synthesized using *Ficus benghalensis*.
Introduction: Synthesis of metal nanoparticles is widely used due to their potential applicability in various areas such as electronics, chemistry, energy and medicine development. To fulfill the growing need of environmentally friendly nanoparticles, researchers are using plant extract for the synthesis of nanoparticles.
Materials and methods: In this report we used the extract *F. benghalensis* to synthesize silver nanoparticles and were characterized using UV- visible spectrometer and were also tested for cytotoxic activity.
Results: These biosynthesized silver nanoparticles showed reduced cytotoxic activity and can be developed as novel medicine against pathogenic oral diseases.
Conclusion: With increasing demand in advancements and diagnosis of treatment modalities, green synthesis of silver nanoparticles using *F. benghalensis* has reduced cytotoxic activity and has wider applications in dentistry.
Keywords: F. benghalensis, cytotoxic activity, innovative technology, green synthesis, silver nanoparticles

1. INTRODUCTION:

Nanotechnology deals with the formation of objects less than 100 nanometers. It has created interest among researchers due to its profound impact on energy, chemical, electronics, space industries and drug/gene delivery (1). Properties of nanoparticles demonstrate enormous applications compared to those of mass materials because of their incredibly small size (2).The metal nanoparticles can be produced using different chemical and physical routes like chemical reduction, electrochemical method and radiation(3). Organic strategies for metal nanoparticles which utilize plant materials are considered as convenient and eco- friendly options in contrast to chemical and physical methods (4).

Biological route of synthesis reduces the generation of hazardous substances to human wellbeing and the environment. With the development in nanotechnology, size controlled blends of different metal and metal oxide nanoparticles can be made. These nanoparticles have properties to meet specific functions (5). Recently researchers have developed interest in the synthesis of silver nanoparticles due to their antimicrobial activity and their use as anticancer agents (6). There are several studies in which silver nanoparticles have been synthesized using natural products like, alfalfa sprouts, and chilli (*Capsicum annuum*), green tea (*Camellia sinensis)* (7-9). Using plant for nanoparticles synthesis is advantageous over other biological processes as it eliminates the elaborate process of maintaining cell cultures (10). It incorporates the therapeutic properties of the plant (11).

Cytotoxicity can be defined as the nature of being harmful to cells. Brine shrimp lethality assay using the larvae of the crustacean, *Artemia salina* is a more common method which is employed to analyse the cytotoxicity of bioactive compounds (12,13). In this study in order to synthesize silver nanoparticles by biological route we have utilized *Ficus bengalensis* (banyan tree) leaf extracts for this purpose in present investigation. *F. benghalensis* belonging to the family Moraceae is a large tree, 20–30 m high, with wide-spreading branches bearing aerial roots. Previous studies reveal hydroalcoholic leaf extracts of *F. benghalensis* reported to have free radical scavenging properties, therefore showed immunomodulatory and antioxidant activity. The present study aims to analyse the cytotoxicity of the silver nanoparticles synthesized using *F. benghalensis.* Our team has extensive knowledge and research experience that has translated into high quality publications (14-32) The present study aims to analyse the cytotoxicity of the *A. webbiana* nanoparticles.

2. MATERIALS AND METHODS

2.1 Preparation of plant extract

The preparation of plant extract was done using *F. benghalensis.* 1 gm of *F. benghalensis* mixed with 100 mL of distilled water and boiled in 60-70 degree celsius in the heating mantle for 10-15 minutes. Add filtered using Whatman no. 1 filter paper. The filtrates were stored at 5°C for further experiments.

2.2 Synthesis of silver nanoparticles (AgNPs) using *F. benghalensis*

1 milli molar of Silver nitrate was dissolved in 90 mL of double distilled water. The *F. benghalensis* extract was added (10 mL) with the metal solution and was made into 100 mL solution. The solution was kept in a magnetic stirrer for nanoparticle synthesis. The colour change was observed visually and photographs were recorded in particular interval. The solution was centrifuged using lark refrigerated centrifuge. The solution was centrifuged at 8000 rpm for 10 minutes and the pellet was collected and washed with distilled water twice. The final purified pellet was collected and dried at 60°C for 24 hours. The final product was stored in an airtight eppendorf tube.

2.3 Conformation of *AgNPs*

The synthesized nanoparticles solution was confirmed by using UV-vis-spectroscopy. 3 mL of the solution was taken in a cuvette and scanned in a double beam UV-vis-spectrophotometer from 300 nm to 700 nm wavelength and the results were recorded.

2.4 Cytotoxic activity of *F.* benghalensis mediated *AgNPs*

Brine shrimp lethality assay was performed for determining the cytotoxic effect of *AgNPs*. Brine shrimp eggs were procured from Aquatic Remedies, Chennai. The hatching of eggs was encouraged by adding it to the artificially made seawater by dissolving 36g of sea salt in 1000 mL of distilled water. This artificial seawater was added to the chamber that had a partition for dark and light areas. Shrimp eggs were added to the dark area of the chamber. Once the eggs hatched, it took 2-3 days for it to mature into larvae. These larvae moved to the light area of the partition. The hatched nauplii was used for the cytotoxicity evaluation. A 6-well ELISA plate was

taken and 10-12mL of artificial seawater was added to each of the 6 wells and 10 nauplii were added to each well. Then, 5 different concentrations of *F. benghalensis* mediated *AgNPs* (5 µl, 10 µl, 20 µl, 40µl, 80µl) were introduced to each of the 5 wells and a control containing only the seawater was taken in one well (Figure 1). The wells were left uncovered under the lamp. The number of surviving nauplii was recorded periodically after 24 h and 48 h of incubation.

3. RESULTS AND DISCUSSION

3.1 Visual observation

In the present study, *AgNPs* that were synthesized using *F. benghalensis* displayed a change in colour from light brown to dark brown within 48 h and did not undergo any further color change indicating the synthesis process is complete (Figure 2). This visual observation of change in colour indicates the complete formation of nanoparticles (33). Previous studies have reported the change in colour during the synthesis process of metal nanoparticles synthesized using *F. benghalensis* (34). It was observed that the colour of silver nitrate solution changed from colourless to brown with increasing intensity with incubation time, indicating formation of silver nanoparticles. It is well known that silver nanoparticles exhibit yellowish brown colour in water (35). The silver nanoparticles mediated by *Allium sativum* indicated color change from light brown to dark brown similar to the present study (36).

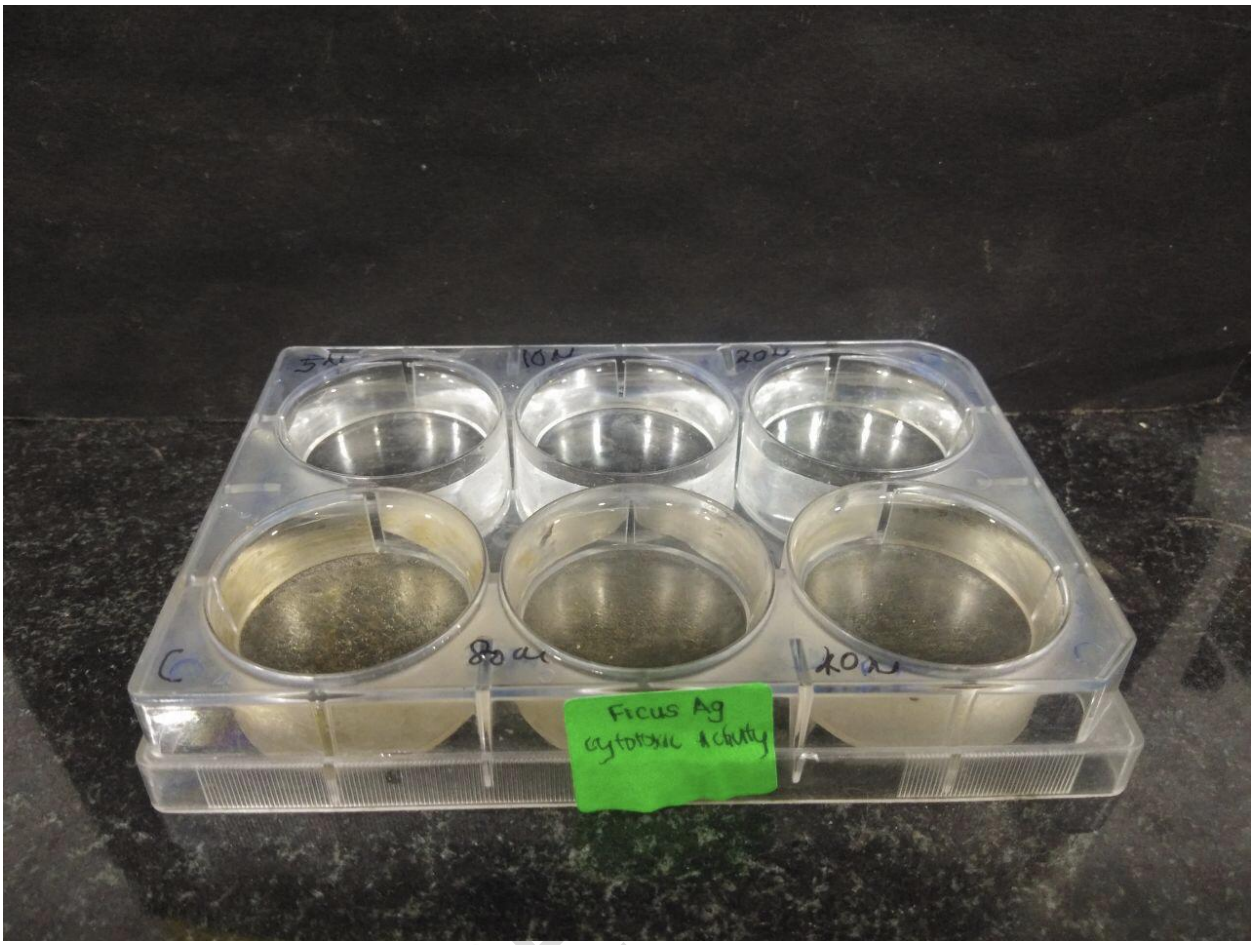

Fig. 1. Brine shrimp lethality assay comprising ELISA plate wells with different concentrations of *F. benghalensis* mediated silver nanoparticles and a control observed for the presence of live nauplii after 24 h and 48 h incubation

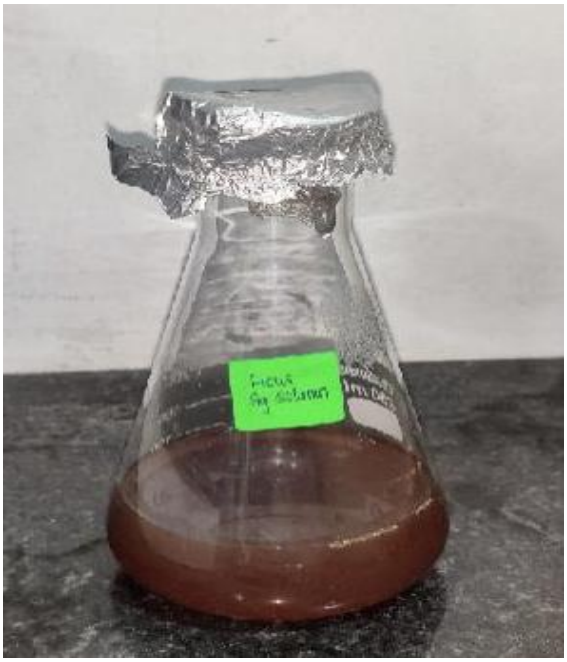

Fig. 2. Reduction of silver ions to silver nanoparticles visually identified by color change

3.2 UV-vis Spectrophotometer Analysis

The UV-vis spectroscopy analysis of the present study showed that the surface plasmon resonance band peak is positioned at wavelength 450 nm (Figure 3). Previous studies confirmed synthesis of *AgNPs* showing similar absorbance pattern. UV–visible spectrum of the aqueous medium containing *AgNPs* synthesized using *Pedalium murex* showed absorption peak at around 430 nm (37). UV visible spectra gave surface plasmon resonance for synthesized *AgNPs* from *Megaphrynium macrostachyum formed* peaks between 400–450 nm (38).

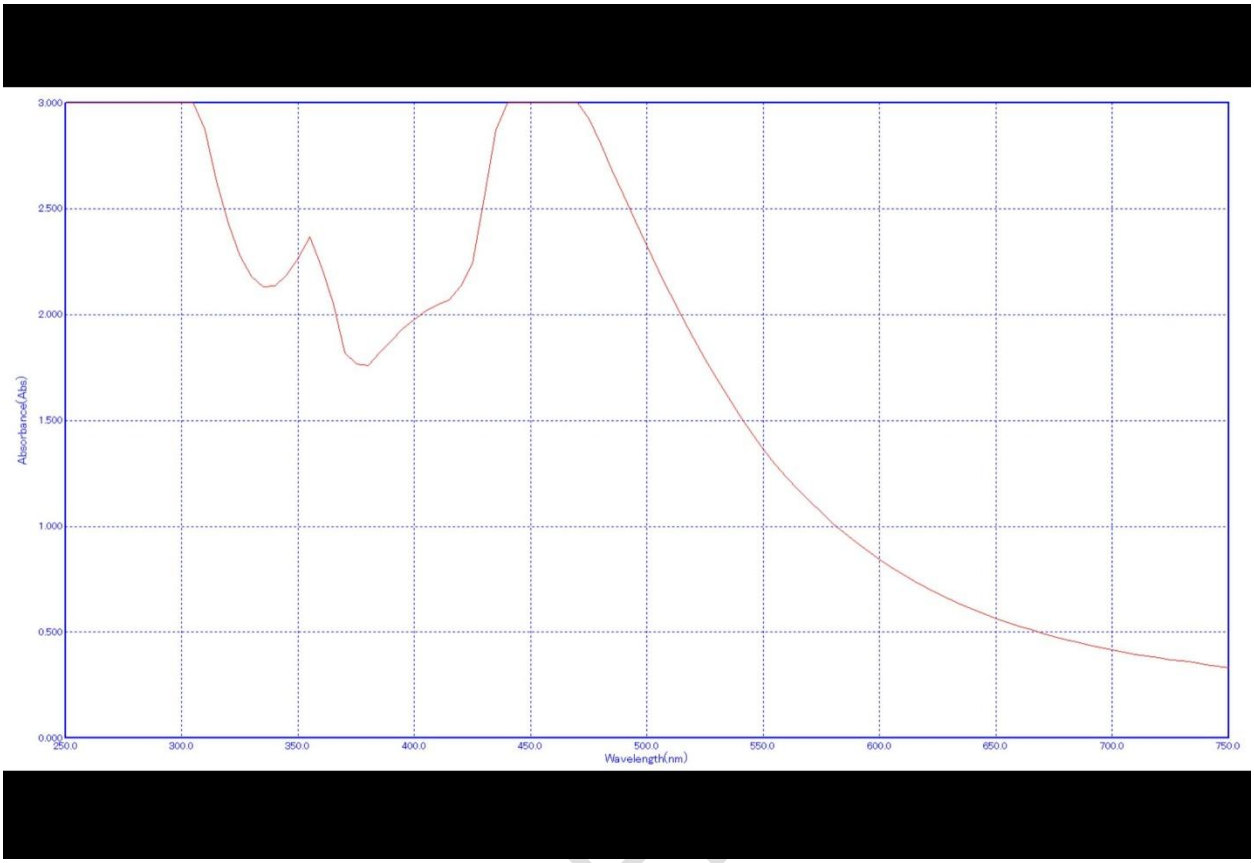

Fig. 3. UV- Vis Spectroscopic analyses of silver nanoparticles synthesized from *F. benghalensis* recorded as function of time

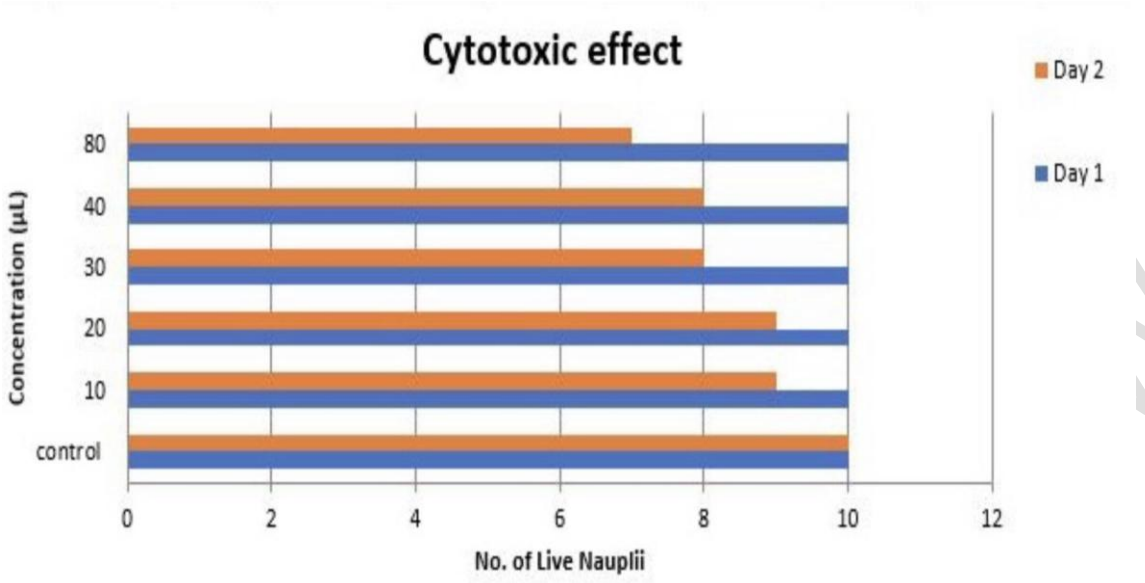

Fig. 4. Cytotoxic effect of *F. benghalensis* at different concentrations compared with standard

3.3 Cytotoxicity Analysis

Brine shrimp lethality assay is an important test in the study of cytotoxicity, that gives us the information about the cytotoxic effect exhibited by a bioactive compound to cells [39]. The viability of the nauplii was analysed for different concentrations of AgNPs that are synthesized from *F.benghalensis* (Figure 4). After 24 hours, 100% of the nauplii were alive in concentrations of 10µl,20 µl,30 µl,40 µl and 80 µl. The control also showed 100% of the nauplii to be alive. After 48 hours,it was found that at a minimal concentrations of 10µl and 20µl 90% of the nauplii and at concentrations of 30µl and 40µl, 80% of the nauplii were alive. At a concentration of 80 µl, 70% of the nauplii were alive. Whereas the control showed 100% of the nauplii to be alive. Thus the increase in the concentration increased the cytotoxicity similar to other studies. AgNPs synthesized using *Bergenia ciliata* showed the cytotoxic effects against brine shrimp (*Artemia salina*) nauplii with a value of 33.92 µg/ml LD_{50}(40). Cytotoxicity of *Ricinus communis* mediated AgNPs showed that the concentration under 20 µg/mL were biologically compatible (41). AgNPs synthesized using *Lantana camara* showed cytotoxic effects on Brine shrimp (*A. salinanauplii*) with LD_{50} value of 514.50 µg/ml (42). Future research in *F. benghalensis* mediated silver nanoparticles evaluating its biological properties like antimicrobial, anti- inflammatory and antioxidant activities can bring about the development of nano-formulations which may act as therapeutics in various diseases.

4. CONCLUSION

Within the limitations of the study we can conclude that *F. benghalensis* enabled the synthesis of stable AgNPs. The results indicate that the AgNPs synthesized from *F. benghalensis* showed reduced cytotoxicity. Thus, various nano-formulations at lower concentrations of these nanoparticles can be developed that are safe, eco- friendly and economical.

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