Research Article

Cytotoxic effect of *Aloe vera* and neem herbal formulation-assisted silver nanoparticles

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ABSTRACT

Aim: The aim of this study was to assess the cytotoxic effects of *Aloe vera* and neem herbal formulation using silver nanoparticles. **Introduction:** The changing lifestyle as well as the food habits has resulted in the introduction of numerous diseases in the past few decades. Moreover, by the passing day, we are becoming more aware about the drug resistance that is developed in most of the disease-causing pathogens. In this scenario, bringing more herbal medicines into the market is the only way to improve the situation. Moreover, neem and *A. vera* already being proven about its antioxidant as well as many other medicinal properties were taken to check the cytotoxic effects along with the help of silver nanoparticles. **Materials and Methods:** In this study we have collected Aloe vera and Azadirachta indica and silver nanoparticles were synthesized using an herbal formulation. Finally the cytotoxic effects of silver nanoparticles investigated using brine shrimp lethality assay. **Results:** The silver nanoparticles can be synthesized using herbal formulation using *A. vera* and *A. indica.* **Conclusion:** The silver nanoparticles synthesized using herbal formulation of *A. vera* and *A. indica* have cytotoxic effects.

KEY WORDS: *Aloe vera, Azadirachta indica*, Cytotoxic effects, Green synthesis, Silver nanoparticles

INTRODUCTION

Metal nanoparticles are gaining prominence due to its unique physiochemical characteristics including catalytic activity, optical properties, and magnetic properties. Silver being a noble metal due to the biocompatibility has attained a lot of interest among the researchers, and it has been used in various medications and is well known for its inhibitory effect toward almost all kind of bacteria including Gram-positive and Gram-negative bacteria.[1,2] Various methods are administered for the synthesis of nanoparticles which include chemical, physical, and ultraviolet (UV) methods. However, the major drawback in their synthesis is the production of hazardous side products when chemical methods are administered.[3,4] Hence, plants are considered as a better platform for the synthesis of nanoparticles since they produce eco-friendly by-products.[5,6]

Indian trees and herbs, especially neem and *A. vera,* have been used form the prehistoric time due to its curative properties and it is an ingredient of various medicines available in the market.[7] Moreover, silver nanoparticles are proved to act as an efficient catalyst.[8] In certain studies, it has been proved that lower nanoparticle concentration when used along with conventional therapeutic materials will give the best results. Moreover, they have found that silver nanoparticles are known to be bactericidal as well as cytotoxic to mammalian cells.[9-11] The nanoparticles may penetrate deep inside the cell wall, disrupting the phosphorus and sulfur compounds such as the DNA and other metabolic proteins present in the cell; also, the nanoparticles will disrupt the cellular permeability as well as the cellular respiration.[12-16]

It has been reported that AE which is a hydroxyanthraquinone which is a naturally found component in the leaves of *A. vera* is a cytotoxically potent agent.[17,18] The damage caused to the cell and tissue due to the free radicals in the body is known as oxidative damage.[19] Moreover, aloe has a large demand in the world market since it can be used as a flavoring liquid and as a raw material for aloin.[20,21]

MATERIALS AND METHODS

Preparation of Plant Extract

Leaves of *A. indica* and *A. vera* were collected from Chennai. The collected leaves were washed 3-4 times

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using distilled water and dried in shade for 7–14 days. The well-dried leaves were then crushed into powder using mortar and pestle. The collected powder of the leaves was stored in airtight containers.

About 1 g of *A. indica* leaf powder was dissolved in distilled water and boiled for 5–10 min at 60–70°C. The same was done for the powder of *A. vera.* The solutions were then filtered using Whatman No. 1 filter paper. The filtered extract was collected and stored at 4°C for further use.

Synthesis of Nanoparticles

About 1 mM of silver Nitrate was dissolved in 90 ml of double distilled water. The plant extracts of *A. indica* and *A. vera* were added with the metal solution, and this solution was made into a 100 ml solution. The color change was observed visually and photographs were taken for the record. The solution was then kept in a magnetic stirrer/orbital shaker for nanoparticle synthesis.

Characterization of Silver Nanoparticles

The synthesis of nanoparticles is primarily characterized using UV-vis-spectroscopy. 3 ml of the solution is taken in a cuvette and scanned in UV-vis-spectrometer under 350 nm–550 nm wavelength. The results were recorded for graphical analysis.

Preparation of Silver Nanoparticle Powder

The Ag-NP solution was centrifuged using Lark refrigerated centrifuge. The centrifugation was done at 8000 rpm for 10 min, and the pellet was collected and washed with distilled water twice. The final purified pellet was collected and dried at 60°C. Finally, the nanoparticles powder was collected and stored in an airtight Eppendorf tube.

Brine shrimp eggs were obtained from the New Aqua Laboratory in Thampanoor, Thiruvananthapuram. Filtered, artificial seawater was prepared by dissolving 36 g of sea salt in 1 L of distilled water for hatching the shrimp eggs. The seawater was put in small plastic container (hatching chamber) with a partition for dark (covered) and light areas. Shrimp eggs were added into the dark side of the chamber while the lamp above the other side (light) will attract the hatching shrimp. 2 days were allowed for the shrimp to hatch and mature as nauplii (larva). After 2 days, when the shrimp larvae are ready, 5 mL of the artificial seawater and 5 mL of nanoparticles solution were added to each test tube, and 10 brine shrimps were introduced into each tube. Thus, there were a total of 30 shrimps per dilution. The artificial seawater up to 10 mL per test tube is control. The test tube was left uncovered under the lamp. The number of surviving shrimp was counted and recorded after 24 h. Using probity analysis, the lethality concentration (LC_{50}) was assessed at 95% confidence intervals. LC_{50} of <100 ppm was considered as potent (active). As mentioned by Meyer and others, LC_{50} value of <1000 µg/mL is toxic, while LC_{50} value of >1000 µg/mL is non-toxic. The percentage mortality (%M) was also calculated by dividing the number of dead nauplii by the total number and then multiplied by 100%. This is to ensure that the death (mortality) of the nauplii is attributed to the compounds present in the nanoparticles.

RESULTS AND DISCUSSION

Visual Observation

The green colour plant extract reacts with silver nitrate solution and change it into brown colour indicates the synthesis of silver nanoparticles shown in Figure 1.

Discussion

The plant extract of *A. vera* and *A. indica* was visually observed [Figure 1]. It was green first when it was added with the metal solution. Then, it turned into brown color extract which was indicated with the formation of silver nanoparticles.[22,23]

UV-vis spectroscopy was used to measure the quantitative formation of silver nanoparticles. The UV-vis spectroscopy analysis with Y-axis having absorbance value and X-axis having the wavelength was plotted in a graph [Figure 2]. A live nauplii of shrimp that has developed from the egg is observed [Figure 3]. The graphical representation of the increasing concentration as well as the number of alive nauplii per day is depicted [Figure 4].

In summary, almost all of the studies cited made a clear picture on the effect of silver nanoparticles on a living cell. Almost all the researchers reported the changes that were morphological due to the introduction of silver nanoparticles by their thorough studies. Moreover, in the recent future, this project can be used as a background for further studies in which more mechanized methods can be used for the

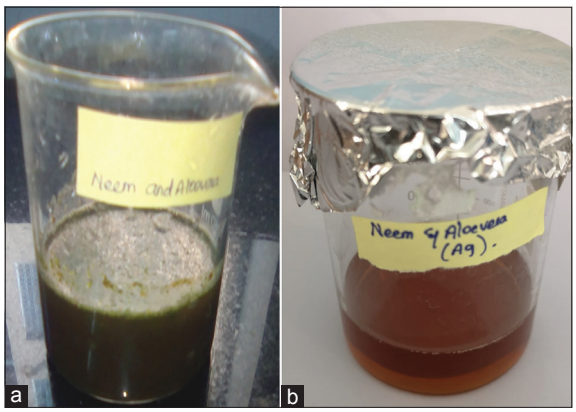

Figure 1: Plant extract and nanoparticles: The green-colored extract turned into brown-colored extract after the addition of metal particles

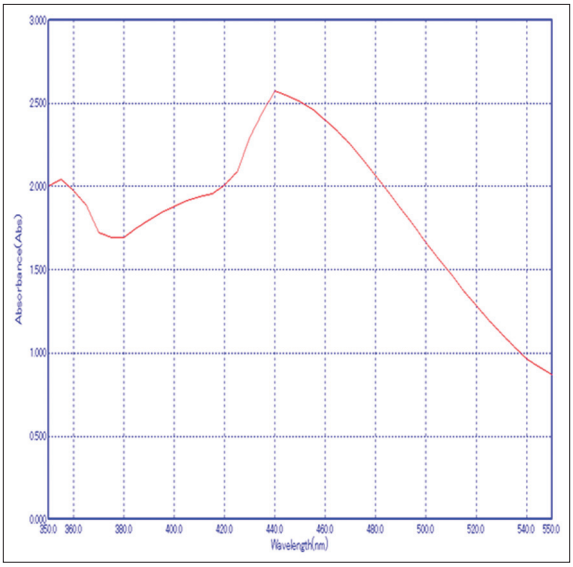

Figure 2: UV-vis spectroscopic analysis of silver nanoparticles. The peak at 440 nm indicates the silver nanoparticles green synthesis

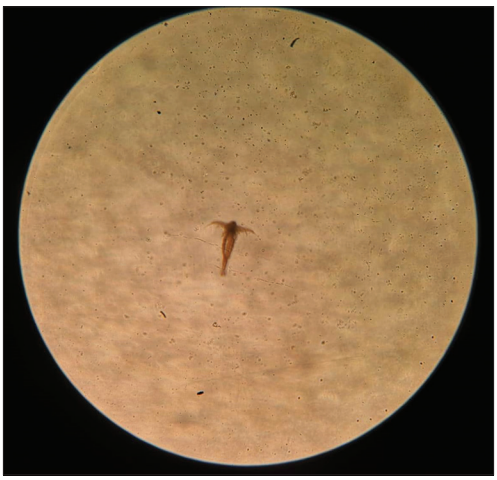

Figure 3: A live nauplii under microscope

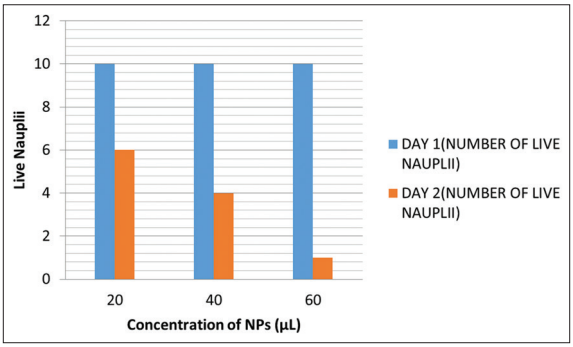

Figure 4: Cytotoxic effect of silver nanoparticles

purification as well as for the identification of active constituents present in *A. vera* and neem.[24]

CONCLUSION

The silver nanoparticles synthesized using the extract of *A. vera* and *A. indica* worked against the shrimp nauplii were used in the study. It was also found that, with an increase in the concentration, more number of nauplii were killed. This indicates that the silver nanoparticles synthesized using the *A. vera* and *A. indica* have some cytotoxic properties, which on doing further research can be used to even rectify the deadly conditions like cancer.

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