Evaluation of Cytotoxic Effect of Silver Nanoparticles Synthesized Using Centella Asiatica

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ABSTRACT:

Centella asiatica is a herb with various medicinal properties used in ayurveda for thousands of years. It is a perennial creeper plant which belongs to the family *Apiaceae.* It is used in the treatment of lupus, leprosy and related skin diseases. Each and every part of the plant can be used in the production of medicines. The present study evaluates the cytotoxic effect of silver nanoparticles synthesised with the help of *Centella asiatica.* Silver nanoparticles were obtained by green synthesis using *Centella asiatica,* where the phytochemicals that are present in the plant helps in the formation of silver nanoparticles. The characterisation of the nanoparticles were done using UV-vis spectroscopy and its cytotoxic effect was analysed using the live nauplii. Cytotoxic effects of silver nanoparticles synthesised using *Centella asiatica* show an increase in concentration, where LD_{50} concentration was 80µl. They help in the treatment of cancer cells. The current study shows that silver nanoparticles synthesised using *Centella asiatica* have a potent cytotoxic activity. The cytotoxic activity is proportionate with the concentration, as the concentration increases the death rate of the cell also increases. Intensified research in this field against a wide range of human cancer cells helps in producing a potent anticancer drug.

Keywords: Bioavailability, cytotoxicity, nauplii, nanoparticles, silver, green synthesis, innovative technology, eco friendly.

1. INTRODUCTION:

Centella asiatica (CA), a creeper belonging to the family Umbelliferae (Apiaceae) is found predominantly in Asian countries growing especially in moist areas up to altitude of about 1800 m. It is found in swamy, moist areas of Asian countries like Pakistan, Sri Lanka, Madagascar, and Republic of South Africa and Pacific Ocean, Japan and Europe (Zweig *et al.*, 2021). Twenty species associated with *Centella asiatica* grow in the tropic or wet pantropic areas and additionally in rocky, higher elevations. It is a tasteless, inodorous plant that thrives in and around water (Kanchi *et al.*, 2019). It has little fan-shaped leaves with white or lightweight purple-to-pink or white flowers and it bears little oval fruit (Lu *et al.*, 2021). It is widely used for treating high pressure, for enhancement of memory (Kumar *et al.*, 2021). Healers from the country of Japan relied on *Centella asiatica* to treat depression that was thought to be a great disorder (Puttarak *et al.*, 2017). Within the Western drugs, throughout the 20th century, *Centella asiatica* and its alcohol extracts reported to have shown positive results in the treatment of Hansen's disease (Bandopadhyay *et al.*, 2023). A nanoparticle is an ultra small particulate matter, which measures

between 1-100 nm in size (Kamath, Nasim and Rajeshkumar, 2020),(Dharsan, Arivarasu and Rajeshkumar, 2020), (Chokkattu *et al.*, 2022). Silver nanoparticle is considered to be an inert, non-toxic and costly material, whose high index of refraction and high capability to soak up lightweight light build it a noteworthy white pigment and environmentally friendly catalyst (Nivethitha, Arivarasu and Rajeshkumar, 2020). The nanosized Ag particles offer white and opacity to products like cream lotions, paints, plastics, papers, inks, food colorants and toothpastes (Weir *et al.*, 2012).

The primary active constituents of *Centella asiatica* are saponins (also known as triterpenoids), that embody asiaticosides, within which a oligosaccharide moiety is coupled to the aglycone asiatic acid, madecassoside and madasiatic acid (James and Dubery, 2009). The triterpene saponins and their sapogenins are responsible for wound healing by inhibiting the assembly of scleroprotein at the wound (Mahmood *et al.*, 2016). Different elements isolated from *Centella asiatica*, like brahmoside and brahminoside, could also be a CNS and uterus relaxant (Gohil, Patel and Gajjar, 2010). Crude extracts of *c.asiatica* contained glycosides isothankuniside and thankuniside showed contraceptive action in mice (Kumar, Kumar and Prakash, 2012). Centelloside and its derivatives are found to be effective within the treatment of blood vessel high blood pressure. Additionally, the whole extract contains plant sterols, flavonoids, and different elements (Gray *et al.*, 2018). In a study, *C. asiatica* was found to have the ability to treat trigeminal neuropathic pain (Wanasuntronwong *et al.*, 2024). Cytotoxicity can be defined as the ability of certain chemicals or compounds to kill or destroy the living cells. MTT assay is the most commonly used assay to evaluate the cytotoxicity of plant extracts against both cancer and normal cell lines (Vairavel, Devaraj and Shanmugam, 2020). Cytotoxicity screening models help in providing preliminary data that can be used to select the plant extracts with significant antineoplastic property that can be used for future work if done(Kaplan and Mehtap Kutlu, 2020). Ethanolic plant extract showed significant effect mainly against the lung and colon cancer cell lines (Rajeshkumar *et al.*, 2020). The chemical constituents of *C. asiatica* like terpenoids and asiaticosides are hydrophobic in nature because of which it shows poor aqueous solubility, extended metabolism and thereby leading to reduced oral bioavailability. Incorporating nanotechnology with the constituents of *C. asiatica* could help in overcoming the above challenges, leading to the production of an efficient drug (Bansal *et al.*, 2024). Silver nanoparticles can be produced from silver nitrate solution, silver nanoparticles were found to have properties like induction of apoptosis, cytotoxicity and gene expression for caspases 3 and 9 (Fard, Tafvizi and Torbati, 2018).

Though various studies have been conducted on these plant extracts based on their physicochemical properties and other effects like antimicrobial activity (Bozkaya *et al.*, 2022), anti-inflammatory activity studies (Mairuae, Cheepsunthorn and Buranrat, 2022) but cytotoxic effects synthesised with Ag nanoparticles were not done. In the present study, the cytotoxic effect of silver nanoparticles synthesized using *Centella asiatica* was explored.

2. MATERIALS AND METHODS:

2.1 Plant Material and extraction:

C. asiatica, also known as gotu kola, was purchased from the neighborhood market and allowed to dry in a shaded area. It was then ground into fine powder. 0.5 g of *Centella asiatica*

was placed in a conical flask with 50 mL of water, labeled, and heated.. The temperature of the heating mantle was set to between 50 and 60 degrees Celsius for this procedure, which took 6 to 8 minutes to finish. A filter paper was then used to filter the mixture. Lastly, the plant extract was made.

2.2 Synthesis of Silver Nanoparticles (Ag NPs):

70 ml of 1 mM of silver nitrate solution in distilled water was mixed with 30 ml of prepared *C. asiatica*. The mixture turned blue. After that, foil paper was used to further block the extract. The process of synthesis started with a uniform dispersion using an orbital shaker, and the solution's color change was periodically monitored. The product was then cooked in a furnace and dried. The resulting annealed product was used as the study's sample.

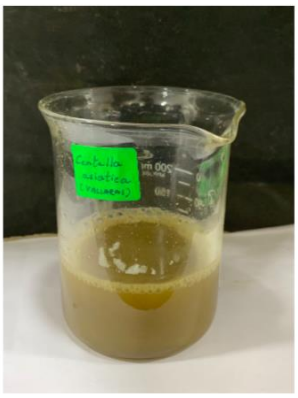

Fig 1: *Centella asiatica*

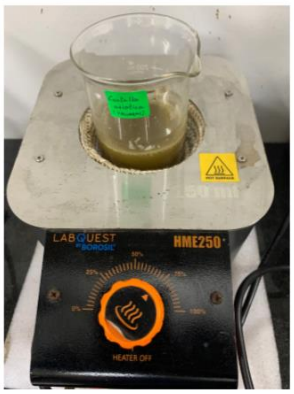

Fig 2: Heated in hot mantle

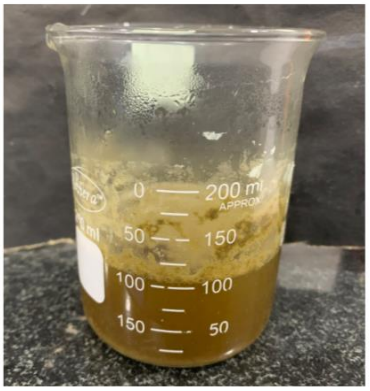

Fig 3: Heated plant extract

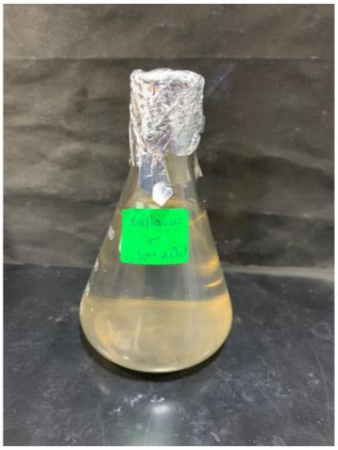

Fig 4: *Centella asiatica* + silver nanoparticles

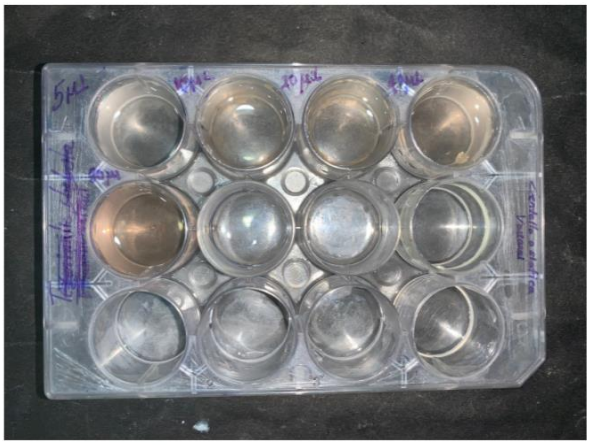

Fig 5: Cytotoxicity assay in ELISA plates with nauplii, nanoparticles assisted with *Centella asiatica*

2.3 Brine Shrimp Lethality Assay:

200ml of distilled water was used to dissolve 2g of iodine-free salt after it had been weighed. Ten to twelve milliliters of saline water were added to six well ELISA plates. In addition, 10 nauplii (5µL, 10µL, 20µL, 40µL, and 80µL) were gradually introduced to each well [Figure 5].Then, in accordance with the concentration level, Ag nanoparticles were added. For a whole day, the plates were incubated. Following a 24 hour period, the ELISA plates were examined

carefully, the quantity of live nauplii present was recorded, and the result was computed using the formula,

$(Number\ of\ dead\ nauplii \times 100) \div (Number\ of\ dead\ nauplii + Number\ of\ live\ nauplii)$

3. RESULTS AND DISCUSSION:

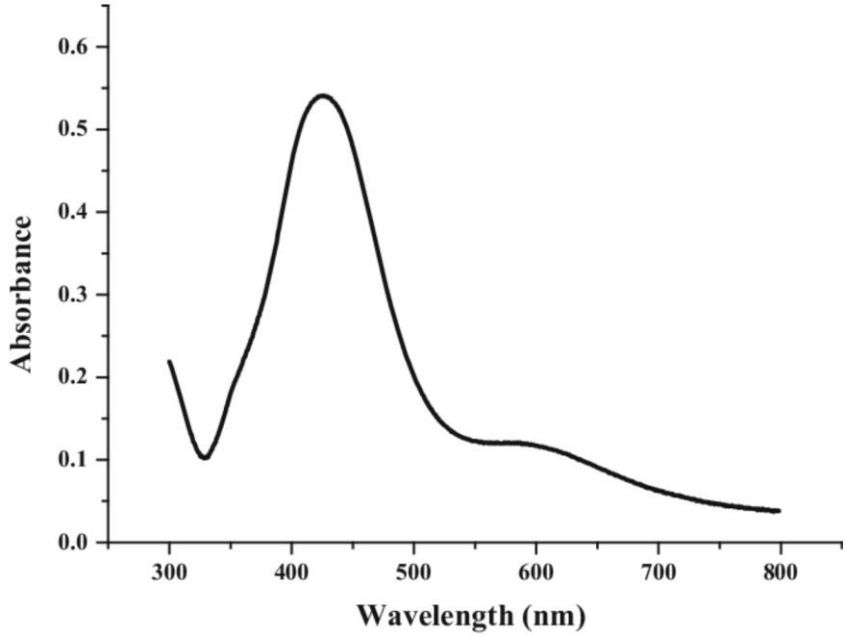

Figure 6: The above graph represents the absorption spectra of silver nanoparticles.

3.1 Characterization of Synthesized Nanoparticles:

Using double beam UV vis spectroscopy, the produced silver nanoparticles were optically measured. The color of the compounds present is directly impacted by absorption spectroscopy in visible wavelengths. The quantitative measurement of various ions, chemicals, and biological macromolecules at various wavelengths is its primary use in analytical chemistry. The produced silver nanoparticles were optically measured at various wavelengths starting at 450 nm (Figure 6).

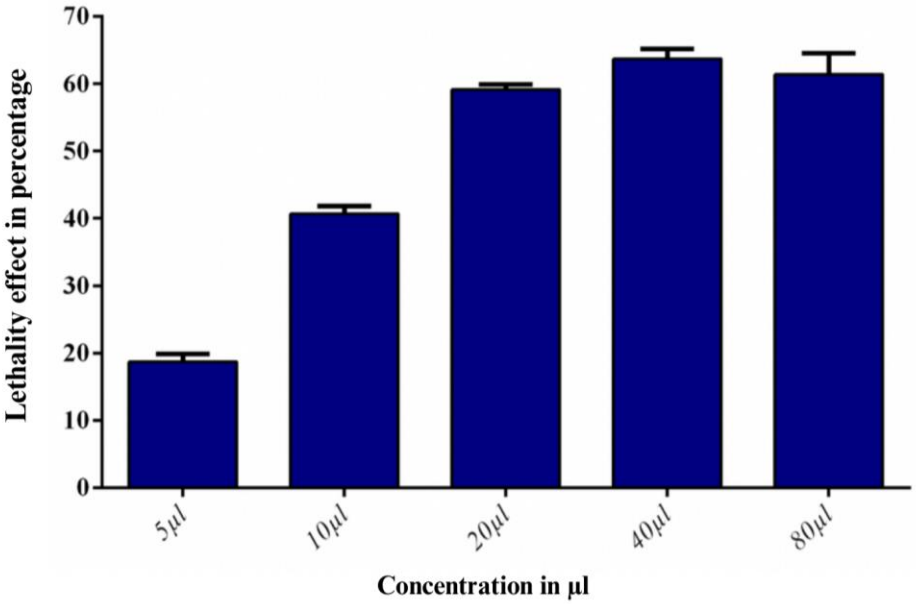

Figure 7: The above graph represents the percentage lethality of silver nanoparticles synthesised using *Centella asiatica*.

Figure 7 shows that when the concentration is 5µl the lethality effect was 20%, in 10µl concentration the lethality was 40% when the concentration was 20µl and it then increases to 60% and 65% when the concentrations are 40µl and 80µl respectively. So the percentage of lethality in X axis is directly proportional to the concentration levels given in Y axis. Brine shrimp lethality assay is one of the simplest method to investigate cytotoxicity as Cytotoxic effects of Ag nanoparticles synthesized using *Centella asiatica* show an incremental pattern with an increase in concentration.The plant contains phytochemicals that are toxic to viable cells. Thus they help in treatment of cancer by preventing increased proliferation of cells.

Plants with significant cytotoxic effects are found useful for development of natural anticancer drugs and agents which have increased greatly during the recent years (Iqbal *et al.*, 2017). Plant derived drugs are mainly used in Unani, Ayurvedic and Homeopathic branches. Many research has been done in the field of nanotechnology due to its various applications in physicochemical, electrical, catalytic, optical, mechanical thermal conduction and antifungal properties (Haridevamuthu *et al.*, 2024),(Bonifácio *et al.*, 2014). Both the oxidant and antioxidant levels in tissues should be balanced (Soyingbe, Mongalo and Makhafola, 2018).

Cancer is said to be a serious malignant disease caused by abnormal cell division and differentiation thereby producing a large number of cells that leads to formation of tumor and later spreads to various body tissues, blood and lymph by obtaining nutrition through the process of angiogenesis (Ghalehbandi *et al.*, 2023). These cancer cells can be killed by inhibiting proliferation and apoptosis inducing agents and effects in the human body (Viswanathan *et al.*, 2019). Phenolic compounds are present in the *Centella asiatica* which are responsible for the plant's cytotoxic effect on the viable and intense proliferating cells (Pittella *et al.*, 2009). According to some research, asiatic acid may cause cancer cells to undergo autophagy by altering the amounts of

certain proteins including p62 and LC3. By combining its inductive impact on Smad7 with naringenin (an Smad3 inhibitor), it can also function as an anti-tumor immunotherapeutic drug (Wiciński *et al.*, 2024). The cytotoxic effect was observed with the *C.asiatica* extract in He-La cells with half-maximal inhibitory concentration (IC50) = 92.8 mL. *C. asiatica* have antiproliferative effects on various tumour cell lines like human colorectal carcinoma, cervical cancer cells and breast adenocarcinoma. The phenolic compounds present in C. asiatica are solely responsible for its cytotoxic effect against tumour cell lines(Rashid *et al.*, 2023). *C. asiatica* shows high cytotoxicity due to the presence of phenols, flavonoids and tannins in leaves and phytochemicals like phytols, sterols and fatty acids. Its antioxidant property helps in reducing premature ageing (Ullah *et al.*, 2020).

In a study, silver nanoparticles were identified to produce cytotoxic effects. The AgNPs has the ability to penetrate into biological barriers. When large amounts of AgNPs accumulate inside the cell, they tend to cause alteration in morphology of monocytes, causing strong damage to extensively proliferating cells (Zhang *et al.*, 2014). In a study, AgNPs has the ability to accumulate in the organs of mice such as spleen, kidney and liver. Silver nanoparticles in an in vivo test done on mice were found to have the ability to cross its blood brain barrier through the circulatory system (Liao, Li and Tjong, 2019). The presence of phenolic compounds is responsible for its lethality and was estimated with nauplii count. The limitations of the current study includes in depth evaluation of only cytotoxic effects in vitro. Further in vivo studies should be undertaken to find the efficacy of the drug as an anticancer agent.

4. CONCLUSION:

The study was done to give pharmacological evidence that the Ag nanoparticles synthesised using *Centella asiatica* have a cytotoxic effect helping in dental and medical applications. Presence of phenolic compounds accounts for its cytotoxicity, which when employed with target assays can be used as an potent anticancer agent. All clinical related aspects must be evaluated and rectification of the shortcomings can develop the plant and its mediated nanoparticle (Ag) as a potent anticancer drug.

5. CONFLICT OF INTEREST:

The author declares that there was no conflict of interest in the present study.

6. ACKNOWLEDGEMENT:

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