Research Article

Anti-cancer and Apoptotic Effects of Silver Nano Particles Infused Green Tea Leaves (*Camellia Sinensis* L) Extracts on HELA Cancer Cell Lines

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Received: 03-02-2026; **Revised:** 27-04-2026; **Accepted:** 05-05-2026; **Published online:** 20-05-2026.

ABSTRACT

Cancer has become one of the most critical health issues, with an increasing incidence and mortality in recent years. Meanwhile, many studies are carried out on discovering new compounds which reflects effective results on cancer cells. Tea comes loaded with antioxidants and compounds like polyphenols and catechins that can fend off free radicals and reduce the risks of chronic diseases. Many tea leaves also come stuffed full of vitamins and minerals along with anti-inflammatory properties and immune system boosters. The green tea leaves were collected and used for preparing green tea leaf extract-mediated silver nanoparticles (GT-AgNPs). The present review highlights examination of the cytotoxic and apoptotic effects on Hela cancer cell lines by using the extract obtained from silver nano particles infused green tea leaves extract. Green tea leaf extract-mediated silver nanoparticles (GT-AgNPs) were synthesized and characterized for their biological and chemotherapeutic activity. The synthesis process involved the reduction of silver ions by green tea leaf extract (GTLE), resulting in the formation of GT-AgNPs. Cytotoxicity assays using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) demonstrated high activity against Hela cancer cells.

Keywords: Green tea, cervical cancer, Hela cell line, Camellia Sinensis L, cell viability, apoptosis, MTT assay.

INTRODUCTION

Tea plant *(Camellia sinensis L.)* is such a source for refreshing and further, it is a popular drink in the world. That is to say, it is all an extract of leaves, leaf nodes, and further as of inter-nodes of the plant *(Camellia sinensis L.)*[1]. They have not only the stimulating effect but also therapeutic properties because of polyphenolic compounds present in them. It seems drinking green tea has indeed benefits in terms of their antioxidant capacities, and such is the case, acting against the free radicals as anti-cancer property.

Cancer preventive activities of green tea and its main constituent, (−)-epigallocatechin gallate (EGCG) have been extensively studied by scientists all over the world. Since 1983, we have studied the cancer chemopreventive effects of EGCG as well as green tea extract and underlying molecular mechanisms. Cancer, caused by the uncontrolled proliferation of cells, is one of our age's most important health problems, and the mortality rate among patients is relatively high [2]. One of the cancer types is cervical cancer, a type of cancer that develops in the cervix, the lower narrow part of the uterus. It is due to the abnormal growth of cells that can invade or spread to other parts of the body. Early on, typically no symptoms are seen. Cervical cancer usually develops slowly over time. Before cancer appears in the cervix, the cells of the cervix go through changes known as dysplasia, in which abnormal cells begin to appear in the cervical tissue. Over time, if not destroyed or removed, the abnormal cells may become cancer cells and start to grow and spread more deeply into the cervix and to surrounding areas. It has been reported by various studies that chemotherapeutic drugs used in cancer treatment cause multi-drug resistance[3,4]. For this reason, the high cost, increasing drug resistance, and side effects of current therapeutic approaches are forcing scientists to explore alternative medicines known as conventional medicine as an option to find new chemicals for cancer treatment. Herbal agents are currently being researched for different uses in many laboratories worldwide. For example, from the point of view of cancer treatment, many researchers worldwide are working on treatment by testing extract samples obtained from different species, such as algae and plants, by different methods in different cancer cell types[5-7].

The present review focuses on the anti-cancer activity and apoptotic effects of green tea leaves extract mediated silver nano particles. It seems apparently that green tea has more health benefits in terms of antioxidant capacity and such a statement can be explained by the fact that each tea is different in terms of composition as well as concentration of antioxidant compounds [8]. Tea is one of the most-widely consumed beverages in the world with a number of different beneficial health effects, mainly ascribed to the polyphenolic content of the tea catechins [9]. No doubt, rutin has performed many pharmacological activities, including antioxidant, cytoprotective, vasoprotective, anticarcinogenic, neuroprotective and cardioprotective activities. The bioactivity of green tea, its benefits is well established and documented. Therefore, this study aimed to unveil the anti-cancer and apoptotic effects of green tea leaves extract mediated silver nano particles on Hela cell lines.

Figure 1: Tea leaves

Figure 2: Green tea Leaves

MATERIALS AND METHODS

Green Tea Leaves

The fresh tea leaves were collected from the hills of Nelyampathy tea estate located in Palakkad, Kerala. The leaf buds used for green tea sampling were properly cut from the tea plants, washed and dried well in shadow. Once the leaves were dry, they were grinded in mortar and pestle to a fine powder. The solvent used was ethanol. Dissolved 2g of powdered sample in 20ml of each solvent, shaken overnight. The next day filtered the final supernatant (the sample or the extract) which was used for silver nano particle synthesis.

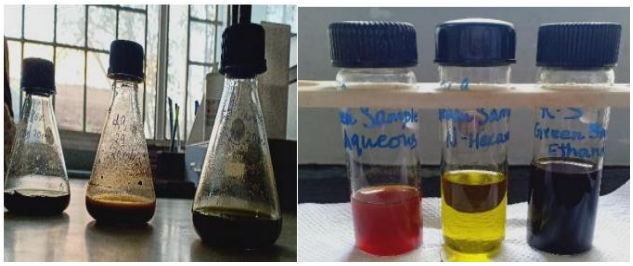


Figure 3: Extract of Green Tea Leaves

Fine grinded tea leaves were soaked in solvents and the extracts were obtained after overnight soaking.

Synthesis of $AgNo_3$ Nanoparticles:

Silver nanoparticles (C. AgNPs) are synthesized by the biological reduction method using extracts from green tea leaves *(Camellia Sinensis)* collected from tea hills at an altitude of 100 m above the ground. The chemicals present in the tea leaf extract act as reducing agents used to reduce Ag+ ions to silver atoms to form C. AgNPs in the solution.

Procedure:

Materials:

Silver nitrate (AgNO3, >99%) and methylene blue dye (C16H18ClN3S, 97%), Sodium borohydride (NaBH4, >99%) and Hydrogen peroxide solution (H2O2, 30% (w/w) in H2O) and the deionized water (DI) with a resistance of 18.2 MΩ was used in all processes. The chemicals were used without any further cleaning. Green tea leaves are not too old and not too young picked from a hill about 100 m above the ground.

Green synthesis of silver nanoparticles from *Camellia Sinensis* leaf extract (C.AgNPs):

Initially, 6 ml of 10 mM AgNO3 solution was slowly added to a volume of the prepared *Camellia Sinensis* leaf extract, under magnetic stirring conditions for about 2 h at a temperature varying between 30 ◦C and 60 ◦C. The resulting solution is yellow-brown once the reaction ends. The solution was centrifuged three times and re-dispersed in water with the same volume for further studies. To know the optimal time for a particle fusion reaction, a certain amount of sample was drawn to investigate the UV–Vis spectrum.

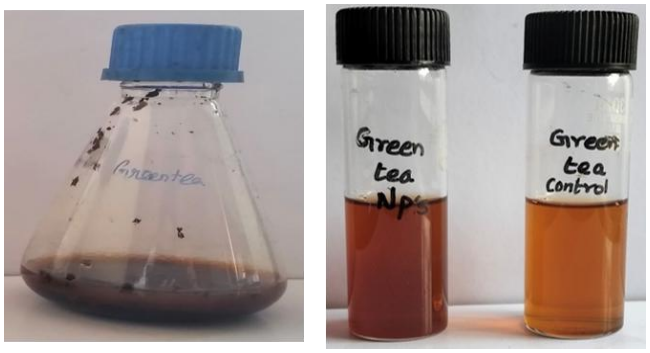


Figure 4: Green synthesis of silver nanoparticles from green tea leaves extract

UV – Visible Spectroscopic Analysis:

Procedure - 0.1µl of C.AgNPs extract was added to 1.9µl of ethanol and centrifuged at 5000rpm for 3mins and the activity was measured under spectrophotometer.

MTT – Cell Viability Assay:

Maintenance of cell lines:

The He La was purchased from NCCS, Pune, India. The cells were maintained in DMEM with high glucose media supplemented with 10 % FBS along with the 1% antibiotic-antimycotic solution in the atmosphere of 5% CO_2, 18-20% O_2 at 37^0C temperature in the CO_2 incubator and sub-cultured for every 2days. Passage No-42 was used for the present study.

Materials:

- Cell lines: He La cell line (From NCCS, Pune)
- Cell culture medium: DMEM high glucose medium (#AL111, Himedia)

- Adjustable multichannel pipettes and a pipettor (Benchtop, USA)
- Fetal Bovine Serum (#RM10432, Himedia)
- D-PBS (#TL1006, Himedia)
- Ethidium bromide-50µg/ml solution, Thermo Fisher, USA
- Acridine orange - 20 µg/ml solution, Thermo Fischer, USA
- Staining solution - 1mlEthidium bromide + 20µlAcridine orangein Media
- Test Compounds: 2 Samples (IC_{50} concentration)
- Cisplatin (Cat No: PHR1624, Sigma)
- 6 well cell culture plate (Biolite - Thermo)
- 50 ml centrifuge tubes (# 546043 TORSON)
- 1.5 ml centrifuge tubes (TORSON)
- 10 ml serological pipettes (TORSON)
- 10 to 1000ul tips (TORSON)

Equipments:

1. Centrifuge (Remi: R-8C).
2. Pipettes: 2-10µl, 10-100µl, and 100-1000µl.
3. Ice bucket with cover. Generally, cells are more stable and tolerate insult better when they're cold. The cover keeps light out, which could bleach the fluorochromes.
4. Fluorescence Microscope –LSM 880 live cell imaging confocal system, Carl Zeiss, Germany.
5. 37°C incubator with humidified atmosphere of 5% CO_2 (Healforce, China).

Steps Followed:

1. Culture cells in a 6-well plate at a density of 2 x 10^5 cells/2 ml and incubate in a CO_2 incubator overnight at 37°C for 24 hours.
2. Aspirate the spent medium and treat the cells with required concentration of experimental compounds and controls, in 2 ml of culture medium and incubate the cells for 24 hours.
3. At the end of the treatment, remove the medium from all the wells and give a PBS wash. Harvest the cells into 2ml eppendorf tubes by trypsinization.
4. Stain cells with 200µl staining solution for 10min. Remove staining solution and wash with PBS to remove excess of dye.
5. Carefully load the 50ul of cell suspension on glass slide and mount it under the cover slip with a drop of mounting medium before imaging
6. Observe under fluorescence microscope with filter cube with Excitation 560/40 nm and Emission 645/75 nm for EtBr and Excitation 470/40 and Emission 525/50 for Acridine orange.
7. Images were overlaid using ImageJ Software v1.48.

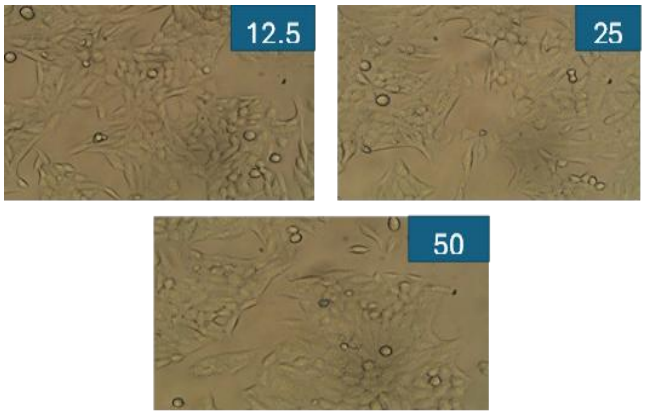


Figure 5: 12.5µg, 25µg and 50µg extract on cancer cell lines

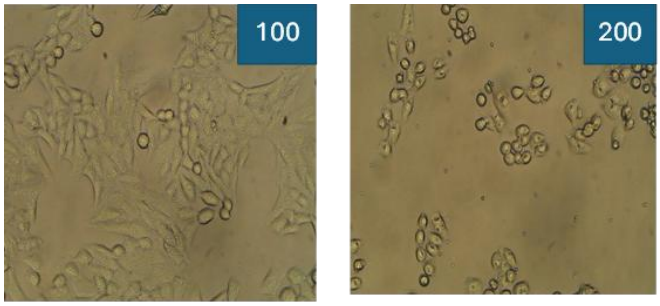


Figure 6: 100µg and 200µg extract on cancer cell lines

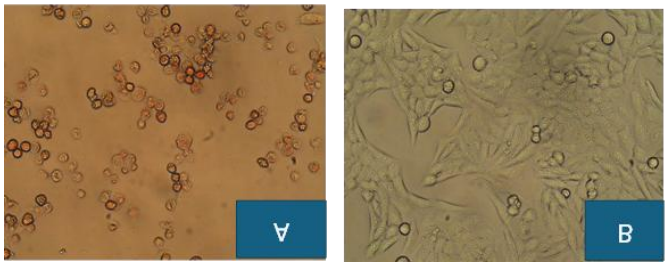


Figure 6: HELA STD Control and HELA Untreated extract on cancer cell lines

RESULTS AND DISCUSSION

UV – vis:

The nanoparticle infused green tea leaves ethanol extract had the Abs value 0.6 is reached. Further, MTT assay was performed on HELA cell lines.

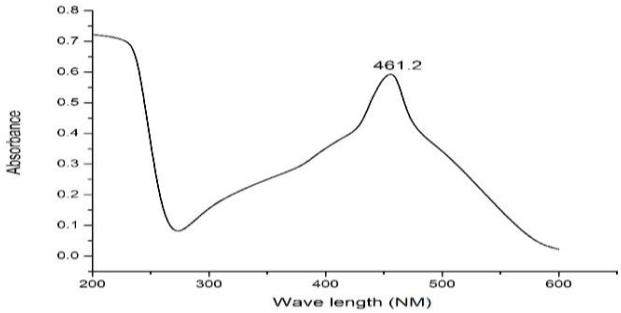


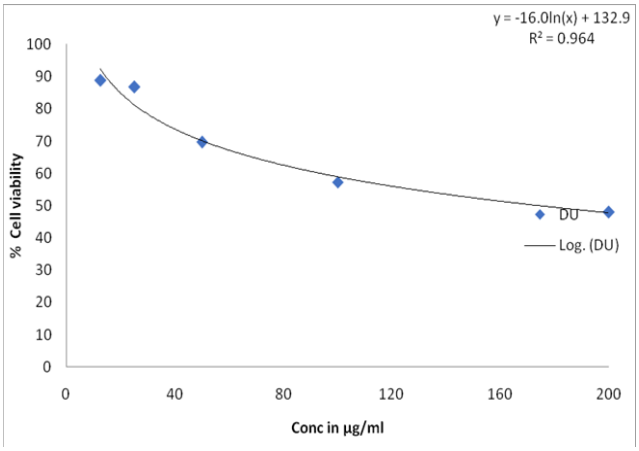
Figure 7: UV – vis AgNPs infused green tea leaves extract

MTT Assay:

Concentration Unit: µg/ml		**Incubation: 24hrs**			**Cell line: HeLa**			**PN-42**
Parameter	**Blank**	**Untreated**	**Std control**	**12.5**	**25**	**50**	**100**	**200**
Abs reading 1	0.047	1.42	0.25	1.252	1.182	0.982	0.812	0.688
Abs reading 2	0.052	1.361	0.199	1.23	1.245	0.986	0.823	0.696
Mean abs	0.0495	1.3905	0.2245	1.241	1.2135	0.984	0.8175	0.692
Mean abs (Sample-Blank)		1.341	0.175	1.1915	1.164	0.9345	0.768	0.6425
Standard deviation		0.041719	0.036062	0.015556	0.044548	0.002828	0.007778	0.00565685
Standard error		0.0295	0.0255	0.011	0.0315	0.002	0.0055	0.004
Cell Viability %		100	13.04996	88.8516	86.80089	69.6868	57.27069	47.912006

IC_{50} value = 124.45ug/ml

MTT Assay Summary-DU	
Condition	**% cell viability**
Untreated	100.00
Std control	13.05
DU-12.5ug	88.85
DU-25ug	86.80
DU-50ug	69.69
DU-100ug	57.27
DU-200ug	47.91



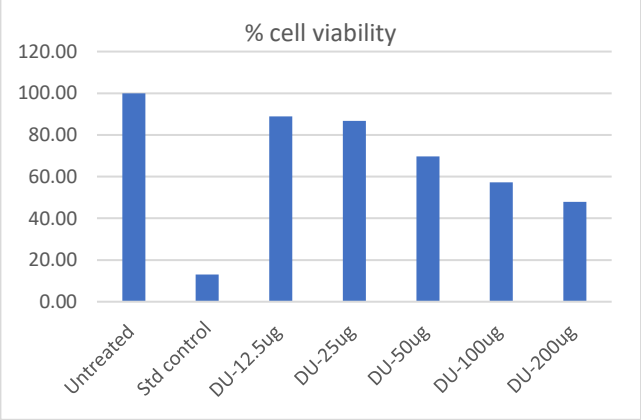


Figure 8: % Cell Viability

Cell Damage:

Plant extract and AgNPs treated with IC50 concentration represented the changes in nuclear morphology of cells. The images were captured at 25x magnification. Bright Green stained nuclei indicates viable cells and Bright Red indicates-dead or damaged cells.

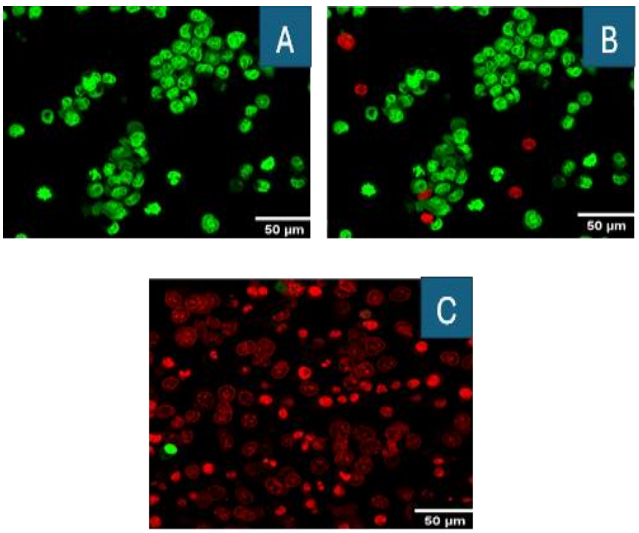


Figure 9: A. Control cells show no DNA damage

B. Plant extract treated cells: (no. of red cells shows changes in nuclear morphology of cells.

C. AgNp treated: Bright Green-stained nuclei indicates viable cells and Bright Red indicates-dead or damaged cells.

CONCLUSION

Overall, in this study, the anti-cancer effect of silver nanoparticles infused Camellia Sinensis L extract was analyzed by using passage no 42 on HeLa cell lines. The tested extract demonstrated anti-cancer effects of plant extract and AgNPs treated with IC50 concentration represented the changes in nuclear morphology of cells with high %cell damage in 200ug extract comparing to other concentrations. The images were captured at 25x magnification. Bright Green stained nuclei indicated viable cells and Bright Red indicated dead or damaged cells. The IC_{50} value was 124.45ug/ml representing that the extracts work on halting cell cycle progression and the activation of apoptotic cell death. All these from the present study suggest that the C.AgNPs extract of green tea leaves extract has the potential for cancer prevention.

Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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