Anti-proliferative and apoptotic effect of papaya leaf and green tea mediated silver nanoparticles using HCT-116 colon cancer cells

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Abstract

Recent advancements in the medicinal use of nano compounds have shown promise for cancer treatment. Nanoparticle-based therapies offer significant advantages, including enhanced biodistribution and precise targeting of cancerous cells. The present study explores the synthesis of silver nanoparticles using bio-assisted methods with green tea and papaya leaf extracts. These green-synthesized silver nanoparticles were subsequently evaluated for their anticancer properties in HCT-116 colon cancer cells. The assessment involved cytotoxicity assays, morphological evaluations, apoptosis induction studies, and biochemical analyses of oxidative stress markers. The findings revealed that HCT-116 cells exposed to papaya leaf and green tea extract-mediated silver nanoparticles (PL-GT-AgNPs) exhibited a significant reduction in cell proliferation, as evidenced by the MTT assay. Notable morphological changes, such as cell shrinkage and membrane blebbing, were observed in cells treated with 20 µg/mL of PL-GT-AgNPs. The dual staining assay further corroborated these apoptotic features. Biochemical analysis indicated a significant increase in lipid peroxide levels, along with a substantial decrease in glutathione (GSH) levels, in the PL-GT-AgNP-treated cells. In summary, PL-GT-AgNPs demonstrates significant anticancer activity against colon cancer cells through several key mechanisms, including inducing cytotoxicity, triggering apoptosis, and modulating oxidative stress.

Keywords: *Silver nanoparticles, green tea, papaya, apoptosis, HCT-116, colon cancer.*

Introduction

Colorectal cancer (CRC) ranks as the third most common cancer worldwide and is a major contributor to mortality, with more than 1.9 million cases and close to 935,000 deaths reported each year [1]. CRC develops gradually, originating from abnormal tissue growths on the inner epithelial linings of the large intestines. When these growths, known as polyps, become cancerous, they can lead to the formation of tumours on the inner walls of the colon or rectum [2]. CRC arises from mutations in oncogenes, tumor suppressor genes, and DNA repair genes, which drive the transformation of normal colon epithelium into adenomas [3]. Surgical resection is the primary treatment for localized colorectal tumors, often followed by chemotherapy, radiotherapy, or targeted/immunotherapy, depending on the tumor stage. Despite a preference for targeted and immunotherapy post-surgery, chemotherapy remains common due to its lower cost and high effectiveness, though it faces issues like tumor resistance and severe side effects [4]. Nanobiotechnology, an emerging field of nanoscience, utilises nano based-systems for various biomedical applications. This rapidly developing field of nanoscience has raised the possibility of using therapeutic nanoparticles in the diagnosis and treatment of human cancers [5].

Among the various metal oxide nanoparticles, silver nanoparticles (AgNPs) have shown exceptional promise and significant results in cancer diagnostics and therapies. This is due to their high surface-to-volume ratio, straightforward synthesis, customizable surface chemistry and functionalization, as well as their effective penetration and traceability within the body [6, 7]. Another benefit of AgNPs is their production through environmentally friendly and practical methods using biogenic agents like plant extracts or other biological materials. Numerous studies have highlighted the biosynthesis of silver nanoparticles (AgNPs) using extracts from a range of biogenic sources, such as plants, microbes, lichens, and fungi, with their biological activities being well-documented [8-10]. Among these methods, plants are preferred over other biological processes because they eliminate the need for maintaining cell cultures. Furthermore, the diverse chemical composition of plant extracts exhibits multifaceted activity, as they can include not only reducing and stabilizing agents but also substances that may inadvertently or intentionally serve as capping agents for AgNPs [11].

Green tea has been widely researched as a therapeutic agent for various cancers, showing promise due to its antioxidant, anti-aging, anti-collagen accumulation, anti-inflammatory, and immune-boosting properties. Green tea has been found to exhibit anti-proliferative and chemo-preventive properties against various types of cancer, including skin cancer [12]. The anti-tumour effects of green tea are largely attributed to catechins, with Epigallocatechin gallate being the most potent among them [13]. *Carica papaya*, has been demonstrated to have significant nutritional benefits. Recent studies have shown potential anti-cancer effects of carica papaya leaf extract mediated nanoparticles against various cancer cell lines viz., MCF-7 cells, A549 cells, HepG2 and DU145 cells [14, 15]. The widespread popularity of green tea and the extensive use of nearly all parts of the papaya tree for their medicinal benefits, combined with the growing interest in green synthesis methods for nanoparticle production, prompted us to explore the effects of silver nanoparticles synthesized with the assistance of green tea and papaya leaf extracts. Given the background, the present study has investigated the effect of green tea and papaya leaf extract assisted silver nanoparticles against colorectal cancer using HCT-116 colon cancer cells.

Materials and Methods

Chemicals and Reagents

DMEM (Dulbecco's Modified Eagle Medium), Phosphate Buffered Saline (PBS), Trypsin-EDTA, Fetal bovine serum (FBS), were purchased from Gibco, Canada. Acridine orange (AO), ethidium bromide (EtBr), Dimethyl sulfoxide (DMSO), [3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyl tetrazolium bromide (MTT), DAPI, AO/EtBr were purchased from Sigma Chemical Pvt Ltd, USA. All other chemicals used were extra pure of molecular grade and were purchased from SRL, India.

Cell line maintenance

HCT - 116 human colorectal carcinoma cells were obtained from the NCCS, Pune with passage number of 18. The cells were grown in T25 culture flasks containing DMEM supplemented with 10% FBS and 1% antibiotics. Cells were maintained at 37°C in a humidified atmosphere containing 5% CO_2. Upon reaching confluency, the cells were trypsinized and passaged.

Cell viability (MTT) assay

The cytotoxic evaluation of papaya leaf and green tea extracts mediated Silver nanoparticles (PL-GT-AgNPs) was evaluated by MTT assay. The assay is based on the reduction of soluble yellow tetrazolium salt to insoluble purple formazan crystals by metabolically active cells. Briefly, $5x10^4$ cells/well were plated in 96 well plates. 24h after plating, the cells were washed twice with 100µl of serum-free medium and starved by incubating the cells in serum-free medium for 3 hours at 37ºC. After starvation, the cells were treated with different concentrations (5, 10, 20, 40, 80 & 160µg/ml) of the PL-GT-AgNPs for 24h. At the end of 20h, the medium from the control and treatment group was discarded and 100µl of MTT containing DMEM (0.5mg/ml) was added to each well. After 4 h of incubation at 37ºC in the CO_2 incubator, the MTT containing medium was discarded and the cells were washed with 1x PBS. The formazan crystals formed were dissolved in dimethyl sulfoxide (100µl) and the intensity of the colour developed was measured using a Micro ELISA plate reader at 570 nm. The number of viable cells was expressed as the percentage of control cells cultured in serum-free medium. Cell viability in the control medium without any treatment was represented as 100%. The cell viability is calculated using the formula: % cell viability = [A570 nm of treated cells/A570 nm of control cells] ×100.

Morphology study

Based on MTT assay we selected optimal doses by linear regression analysis for further studies. Analysis of cell morphology changes was observed using a phase contrast microscope. 2×10^5 cells were seeded in 6-well plates and treated with the highest concentration of 80µg/ml of nanoparticles for 24h. At the end of the incubation period, the medium was removed and cells were washed once with a phosphate buffer saline (PBS pH 7.4). The plates were observed under a phase contrast microscope for morphological changes.

Determination of mode of cell death by acridine orange (AO)/ethidium bromide (EtBr) dual staining

The apoptotic effect of PL-GT-AgNPs nanoparticles in HCT - 116 colon cancer cell death was determined by AO/EtBr dual staining assay. The cells were treated with 80µL of the synthesised nanoparticle for 24h and then the cells were harvested, washed with ice-cold PBS. The pellets were resuspended in 5µl of acridine orange (1mg/mL) and 5µl of EtBr (1mg/mL). The induction of apoptotic in the cells were then observed in stained cells using an inverted fluorescence microscope.

Evaluation of oxidative and anti-oxidative markers

The detection of ROS-related markers, including lipid peroxide (LPO) and reduced glutathione (GSH), was performed using the methods described by Ohkawa et al. [16] and Ellman method [17], respectively.

Statistical analysis

All data obtained were analysed by One way ANOVA followed by Student's t-test using SPSS software. Data were represented as mean ± SD for triplicates. The level of statistical significance was set at $p<0.05$.

RESULTS

Cytotoxicity evaluation by MTT assay

In the present study, the anti-cancer potential of green synthesized PL-GT-AgNPs was evaluated by MTT assay. HCT-116 cells treated with PL-GT-AgNPs exhibited significant inhibition of cell proliferation, as determined by the MTT assay. This anti-proliferative effect was dose-dependent, with the most pronounced inhibition observed at a concentration of 160µg/ml, where only 13% of the cells remained viable, indicating an 87% reduction in cell viability. The IC_{50} was found to be 20.12µg/ml.

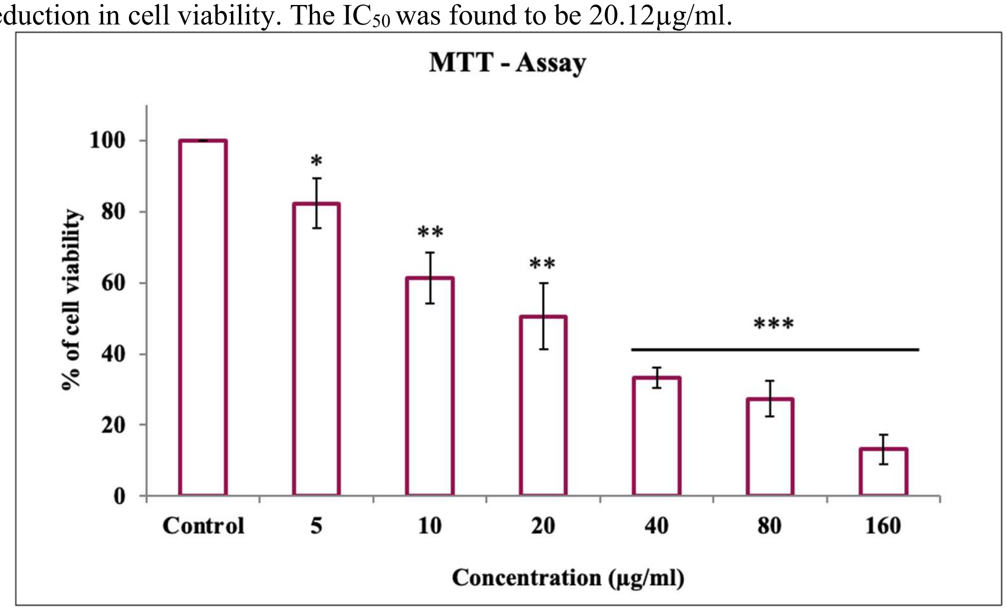
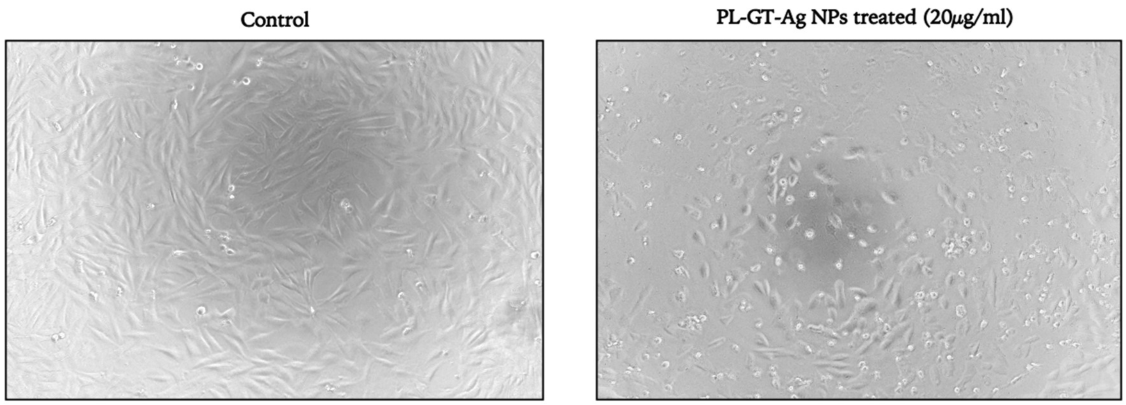


Figure 1: Bar graph showing the cytotoxic effect of papaya leaf and green tea extracts mediated Silver nanoparticles against HCT - 116 human colorectal carcinoma cells. Results are expressed as Mean±SEM (n=3).

Morphological alterations induced by PL-GT-AgNPs

To further assess the anti-cancer effects of PL-GT-AgNPs, cells treated with 20µg/ml of the nanoparticles were evaluated for morphological changes. The images revealed characteristic signs of cell death, including cell rounding and membrane blebbing.

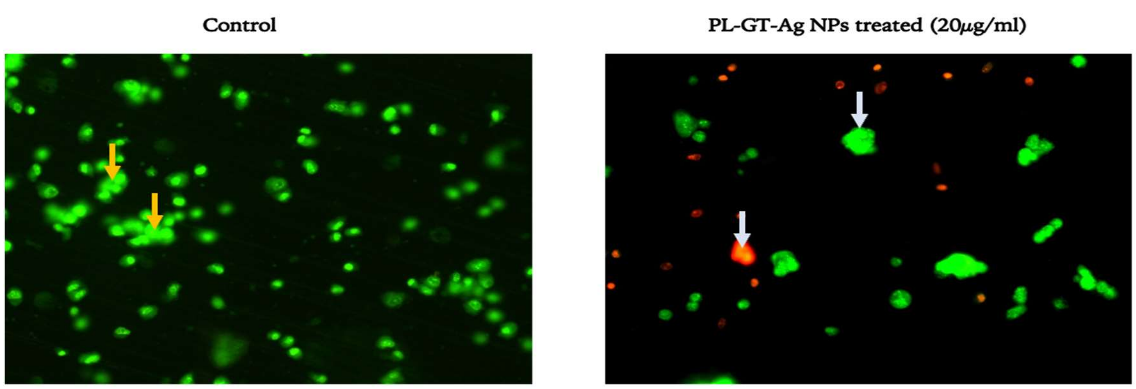


Cells were treated with papaya leaf and green tea extracts synthesized silver NPs (20 µg/ml) for 24 h along with control group. Images were obtained using an inverted phase contrast microscope.

Figure 2: Representative images showing morphological alterations induced by papaya leaf and green tea extracts mediated Silver nanoparticles in HCT-116 human colorectal carcinoma cells, magnification 10x.

Apoptosis effect of PL-GT-AgNPs in HCT-116 cells

To assess the anti-cancer mechanism of PL-GT-AgNPs, cells were treated with 20 µg/ml of AgNPs and stained using the dual EtBr/AO staining method. In the untreated control group, green fluorescence was observed, signifying viable cells, while the PL-GT-AgNPs treated group exhibited membrane blebbing and reddish-orange fluorescence, indicating late-stage apoptosis.



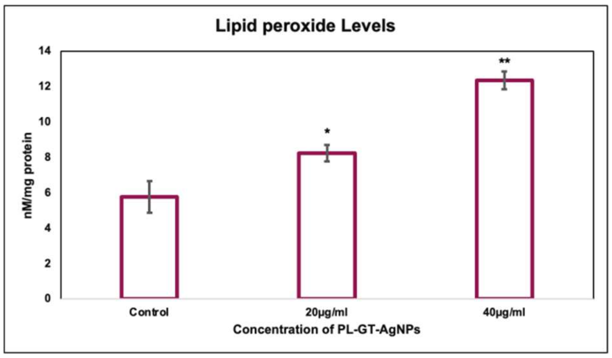
Cells were treated with PL-GT-Ag NPs (20 µg/ml) for 24 h along with control group. Live cells (orange arrows) stained with ethidium bromide and appears green in fluorescence. Early apoptotic cells (Blue arrows) stained by acridine orange appears yellowish green in colour. Images were obtained using an inverted fluorescent microscope.

Figure 3: Representative images showing apoptotic effect of papaya leaf and green tea extract mediated

silver nanoparticles in HCT – 116 human colorectal carcinoma cells by AO/EtBr dual staining assay, magnification 10x

Estimation of oxidative and anti-oxidative markers

To investigate the role of oxidative stress induced by PL-GT-AgNPs in HCT-116 cells, cells were treated with 10µg/mL and 20µg/mL concentrations of PL-GT-AgNPs for 24 hours. Following treatment, lipid peroxidation (LPO) as a pro-oxidant marker and glutathione (GSH) as an antioxidant marker were assessed. The results demonstrated a significant increase in LPO levels, indicating elevated oxidative stress, alongside a marked reduction in GSH levels, suggesting a depletion of the cellular antioxidant defense, compared to the control group.



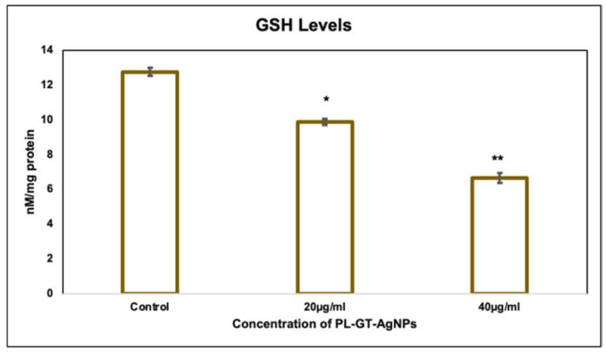


Figure 4: Bar showing the effect of PL-GT-AgNPs on levels of lipid peroxides and reduced glutathione in HCT-116 colon cancer cells. Results are expressed as Mean±SEM (n=3). *, ** indicates significance levels of p<0.05 and p<0.01 respectively Vs untreated control.

DISCUSSION

Previous studies have highlighted the potential of biogenic AgNPs in treating cancer and microbial infections, due to their unique characteristics that have been the determining factors for their biological activities [18-21]. Additionally, papaya leaf extract is rich in bioactive compounds such as alkaloids, glycosides, tannins, saponins, and flavonoids, which contribute to its therapeutic properties [12, 13]. Green tea and its polyphenols (GTPs) are also well-documented for their ability to suppress carcinogenesis and inhibit malignant progression in various cancer types [14, 15]. Building on this foundation, our study demonstrated a significant anti-cancer effect against colorectal cancer, as evidenced by cytotoxicity assays, morphological changes, apoptosis induction, and biochemical analysis of oxidative stress markers. The study was carried out using HCT - 116 colon cancer cells.

Apoptosis is a critical cellular process that regulates cell populations and maintains organismal homeostasis. It is marked by specific morphological changes, including cell shrinkage, chromatin condensation, membrane blebbing, DNA fragmentation, and the formation of apoptotic bodies [22, 23]. In the current study, HCT-116 cells treated with PL-GT-AgNPs showed a significant reduction in cell proliferation, as demonstrated by the MTT assay. Additionally, morphological changes were evident in cells treated with 20 µg/mL of PL-GT-AgNPs. The dual staining assay further confirmed characteristic features of apoptosis, such as cell shrinkage and membrane blebbing. These observed anti-proliferative and pro-apoptotic effects of the synthesised silver nanoparticles in HCT-116 cells align with previous studies, which have reported the cytotoxic effects of AgNPs synthesised from various plant sources on colon cancer cells, HepG2 cells, breast cancer cells and HeLa cells. [24-27].

Previous studies have suggested that the effects of silver nanoparticles (AgNPs) may be linked to their capacity to generate reactive oxygen species (ROS) within cells, leading to oxidative stress (OS). This oxidative stress can disrupt cellular functions such as autophagy and potentially induce cell death [28-30]. In this study, we assessed the levels of oxidative and antioxidative markers—lipid peroxides and reduced glutathione (GSH)—in HCT-116 cells treated with or without PL-GT-AgNPs. The results revealed a significant increase in lipid peroxide levels, accompanied by a notable decrease in GSH levels in the PL-GT-AgNP-treated group. Numerous earlier studies on various metallic nanoparticles have demonstrated that the excessive production of ROS and reactive nitrogen species (RNS) can lead to irreversible DNA damage, mainly through the accumulation of oxidative stress markers [11, 31, 32]. The observed reduction in GSH levels may be due to its conversion to oxidized glutathione, driven by oxidative stress [33]. Additionally, the damage observed may be influenced by the phytochemicals coating the nanoparticles, which facilitate their cellular entry. This enhanced uptake is likely due to the high biocompatibility of biogenic nanoparticles with biological systems [34]. Supporting this hypothesis, our study found significant changes in lipid peroxide and GSH levels in HCT-116 cells treated with green tea and papaya leaf extract bio-assisted AgNPs.

CONCLUSION

Our findings suggest that bio-assisted silver nanoparticles synthesized from *Carica papaya* leaves and green tea significantly reduced the viability of HCT-116 human colorectal cancer cells. The PL-GT-AgNPs demonstrated promising anti-proliferative effects and induced apoptosis in these cancer cells. However, to fully unlock their potential as a chemopreventive agent, further studies are needed to isolate the specific active flavonoids and elucidate the molecular mechanisms underlying their effects. This will provide a deeper understanding of the bioactive components responsible for the observed anti-cancer properties of the *Carica papaya* leaves and green tea-mediated silver nanoparticles.

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CONFLICT OF INTEREST

The authors declared no conflict of interest pertaining to the study.

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