Evaluation of the Phototoxic effect of Chemically Synthesized Silver Nanoparticles on Breast Cancer Cells

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Abstract. Due to their multifaceted biological activity, including reactive oxygen species (ROS) generation, interference with cellular metabolism, and potential to overcome multidrug resistance, silver nanoparticles (AgNPs) have been increasingly investigated for their role in anticancer strategies. Beyond their intrinsic cytotoxicity, AgNPs also exhibit utility in drug delivery and cancer diagnostics. Notably, their ability to convert absorbed light into heat enables targeted photothermal therapy. This study evaluates the chemotoxic and phototoxic effects of chemically synthesized AgNPs on MCF-7 human breast cancer cells. AgNPs were synthesized via chemical reduction and characterized using UV–Vis spectroscopy, dynamic light scattering (DLS), and Zetasizer analysis. The resulting nanoparticles displayed a surface plasmon resonance (SPR) peak at 401 nm, an average hydrodynamic size of 119.3 nm, zeta potential of −30.8 mV, and a polydispersity index (PDI) of 0.269, indicating good colloidal stability. MTT assays showed dose-dependent cytotoxicity, with the IC_{50} value decreased from 7.3 µg/mL under dark conditions to 4.3 µg/mL following 405 nm (5 J/cm²) laser irradiation. Photothermal assessment revealed a ~3 °C temperature increase in AgNP suspensions compared to a negligible rise (~1 °C) in the control, confirming their photothermal conversion efficiency. Morphological changes observed via bright-field microscopy further supported AgNP-induced cell damage. These findings suggest that enhanced cytotoxicity results from a combination of localized hyperthermia and ROS production, highlighting the synergistic photothermal and photodynamic properties of AgNPs. Overall, this study supports the potential of citrate-borohydride synthesized AgNPs as effective photo-responsive agents for targeted breast cancer therapy.

1 Introduction

Breast cancer continues to rank among the most prevalent forms of cancer and remains a major contributor to cancer-related deaths among women across the globe [1]. Across Africa, it makes up nearly 17% of all female cancers and is responsible for about one in five cancer-related deaths among women. In South Africa, more than 9,200 new cases are recorded each year, with an incidence rate of 50.8 per 100,000, making it the most common cancer among South African women [2, 3]. While conventional treatment strategies such as chemotherapy and radiotherapy have led to improvements in patient prognosis, they are still hindered by several limitations. These include systemic toxicity, lack of selectivity, and the eventual emergence of therapeutic resistance [4, 5]. Considering these challenges, nanotechnology has emerged as a transformative tool in the field of oncology, offering enhanced specificity and therapeutic precision. Among the various nanomaterials developed, AgNPs have attracted substantial interest due to their distinct physicochemical characteristics and inherent cytotoxic

capabilities toward cancer cells [6]. The selective accumulation of nanoparticles within tumour tissue is largely facilitated by the Enhanced Permeability and Retention (EPR) effect, which arises from the leaky vasculature and poor lymphatic drainage commonly found in solid tumours, thereby promoting preferential uptake of AgNPs at the tumour site [7]. The antitumor potential of AgNPs is mainly linked to their ability to induce Reactive Oxygen Species (ROS), impair mitochondrial dynamics, and disrupt essential cellular signaling cascades [8]. Additionally, AgNPs serve as versatile carriers for anticancer drugs, contributing to better solubility, improved bioavailability, and more efficient targeted delivery [9].

In addition to their inherent cytotoxic properties, AgNPs exhibit photo-responsive properties. Their strong surface plasmon resonance (SPR) allows absorption of specific light wavelengths, converting them into localized heat, a plasmonic photothermal effect [10]. Light activation also enhances ROS generation, eliciting a photodynamic effect that intensifies oxidative stress in cancer cells [11]. These dual mechanisms make AgNPs promising anti-cancer agents for minimally invasive cancer therapies. Their efficacy, however, is influenced by factors such as particle size, surface charge, and colloidal stability, which are largely dictated by synthesis methods. Chemical reduction using sodium borohydride remains a widely adopted approach for generating stable, well-defined AgNPs [12]. As nanotechnology converges with phototherapy, the development of light-activated AgNP systems represents a growing frontier in cancer treatment, warranting further investigation into their physicochemical-biological interactions and therapeutic optimization. This work aims to evaluate the chemotoxic and phototoxic effects of chemically synthesized AgNPs on MCF-7 breast cancer cells, with a focus on their photothermal and photodynamic contributions under 405 nm laser irradiation.

2 Materials and Methods

2.1 Synthesis of AgNPs

AgNPs were synthesized via chemical reduction method [13]. Briefly, a 10 mL solution of 1 mM silver nitrate ($AgNO_3$) was prepared and heated to 45–50 °C under stirring (500 rpm), followed by the addition of 1 mL of 1 mM sodium citrate ($Na_3C_6H_5O_7$) as a reducing agent. Subsequently, 2–3 drops of 0.1 M sodium borohydride ($NaBH_4$) were added dropwise as a coalescence. The reaction was stirred vigorously for 5 minutes, with a yellowish-brown colour change confirming AgNP formation. The newly synthesised AgNPs were centrifuged to collect them in pellets, dried, and weighed. AgNPs dissolved in distilled water were stored at 4 ºC for further analysis and experiments.

2.2 Characterization of AgNPs

The synthesized AgNPs were characterized to verify formation and evaluate physicochemical properties. UV–Vis absorption spectra were recorded between 300–600 nm using a VICTOR Nivo™ multimode plate reader (PerkinElmer) to confirm SPR. Particle size, polydispersity index (PDI), and zeta potential were measured using a Zetasizer Nano ZS (v7.10, Malvern Instruments, UK) at a fixed scattering angle and controlled temperature.

2.3 Photothermal Effect Measurement

Photothermal activity was examined by dissolving 10 mg of freeze-dried AgNPs in 2 mL of Milli-Q water to yield a 5 mg/mL suspension. This solution was placed in a sterile 35 mm petri dish, while a control containing only 2 mL of Milli-Q water was prepared in parallel. Both samples were irradiated with a 405 nm blue diode laser at an energy fluence of 5 J/cm². Surface temperature was monitored using an infrared thermal imaging camera at the start of irradiation and after 5 minutes of continuous laser exposure.

2.4 Cell Culture

The human breast adenocarcinoma cell line MCF-7 (ATCC® HTB-22™) was procured from the American Type Culture Collection (ATCC) and maintained under standard culture conditions in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (Pen-Strep). Cells were maintained at 37 °C in a humidified incubator under 5% CO_2 atmosphere and sub-cultured upon reaching 70–80% confluency.

2.5 Treatment

To evaluate the chemo and phototoxic effects of AgNPs, MCF-7 cells were seeded in 96-well plates and treated with AgNP concentrations of 1.25, 2.5, 5, 10 and 20 µg/mL for 24 h. Phototoxicity was evaluated by treating cells with the same AgNP concentrations for 24 h, followed by irradiation using a 405 nm blue light diode laser

at a light dose of 5 J/cm², with intensity maintained between 22 and 23 mW/cm². Control groups included an untreated group (without AgNPs treatment and irradiation) and a light control group (without AgNPs treatment).

2.6 MTT Cell Viability Assay

Cell viability was evaluated using the MTT assay (Cell Proliferation Kit I, No. 11465007001, Invitrogen™), which measures mitochondrial-dependent conversion of MTT into insoluble formazan by metabolically active cells. Post treatment and incubation period, 10 µL of the MTT labelling reagent was added to each well and cells were incubated at 37 °C for 3 h. Formazan crystals were dissolved using 100 µL of Solubilization solution into each well, plates were made to stand overnight in the incubator, and the optical density at 540 nm was recorded using a VICTOR Nivo™ (PerkinElmer) multimode plate reader. Viability was calculated relative to untreated controls.

2.7 Morphological Analysis

Morphological changes were assessed using bright-field microscopy. MCF-7 cells treated with IC_{50} concentrations of AgNPs with and without irradiation were imaged alongside untreated groups using a Wirsan Olympus CKX 41 inverted microscope with an Olympus C5060-ADUS camera equipped with CellSens software (v2.3).

2.8 Statistical analysis

All experiments were conducted in triplicate and repeated independently. Differences between the treated and control groups were analyzed using the Student's t-test to determine statistical significance. Statistical analysis was performed using SigmaPlot version 14.0, with results reported as mean ± SD. Significance levels were indicated as *p < 0.05, **p < 0.01, and *p < 0.001.

3 Results

3.1 Characterisation of AgNPs

Fig. 1a shows that the formation of AgNPs through UV–Visible absorption spectroscopy with a distinct SPR peak at 401 nm. As shown in Fig 1b and Fig 1c, DLS analysis showed an average hydrodynamic diameter of approximately 119.3 nm, a zeta potential of −30.8 mV, and a PDI of 0.269 at pH 7.4. These values indicate good colloidal stability and uniform particle distribution under physiological conditions.

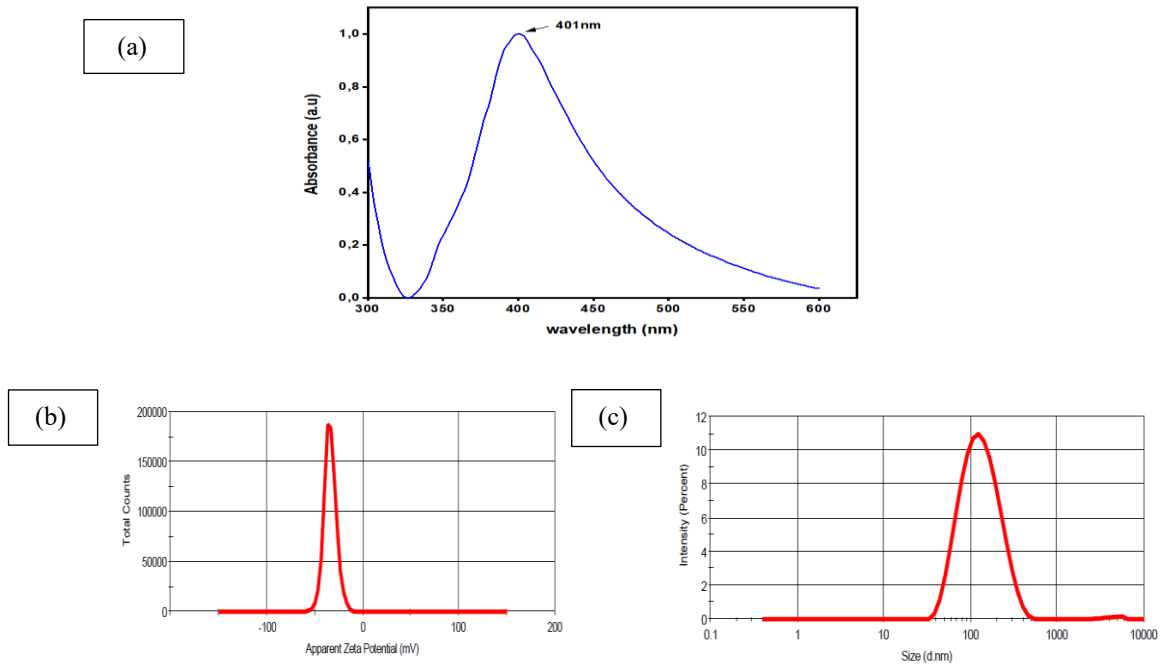

Figure 1. (a) UV–Visible absorption spectrum for AgNPs, (b) zeta potential, and (c) particle size.

3.2 Photothermal Activity of AgNPs

Fig. 2 shows the thermal response of AgNP suspensions and water (control) following 5 minutes of irradiation with a 405 nm laser at an intensity of ~21.94 mW/cm^2. The AgNP-treated sample exhibited a temperature increase of approximately 2.8 °C, while the water control showed a minimal rise of ~0.9 °C. This indicates the moderate photothermal conversion ability of AgNPs under light exposure.

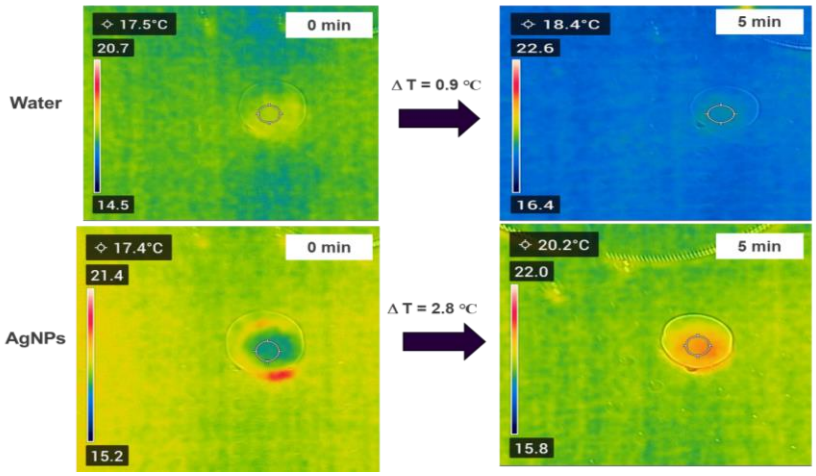

Figure 2: Thermal images of irradiated water and AgNPs solution at 0 and 5 min.

3.3 Chemotoxic and Phototoxic Effects on MCF-7 Cells

MCF-7 cells treated with AgNPs exhibited a concentration-dependent decrease in cell viability in both unirradiated and irradiated groups (Fig. 3a). The half-maximal inhibitory concentration (IC_{50}) for AgNPs in the absence of irradiation (chemotoxicity) was determined to be 7.3 µg/mL. Upon irradiation, the IC_{50} dropped to 4.3 µg/mL, indicating a significant enhancement in cytotoxicity when combined with phototherapy (Fig. 3b). This reflects an approximate twofold increase in cell death, suggesting the additive or synergistic contribution of light-induced cytotoxicity.

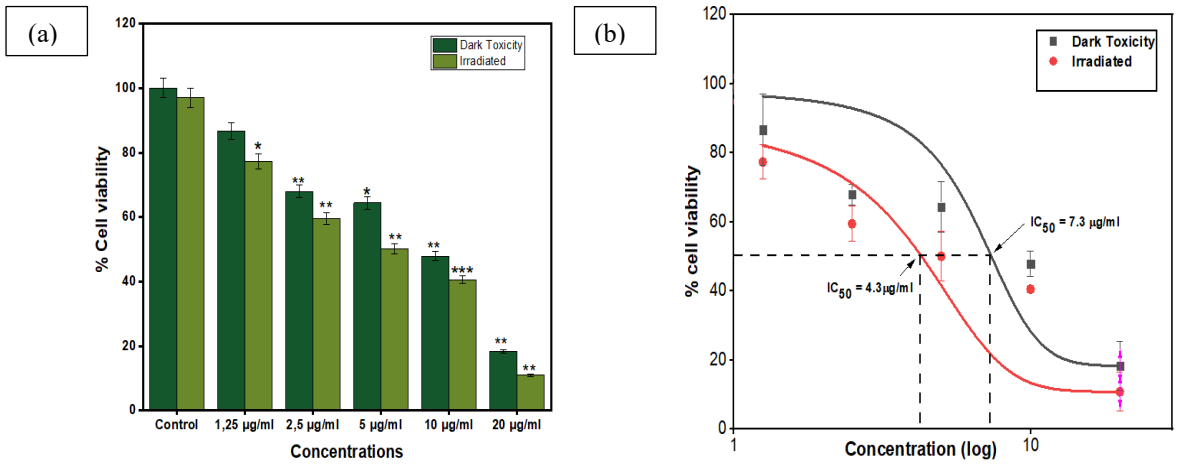

Figure 3. (a) Percentage cell viability of MCF-7 cells treated with AgNPs with and without light irradiation determined by MTT assay post 24 h, (b) sigmoidal concentration-response curve of both treatment groups. IC_{50} values are indicated for both treatment groups. Data points in the graphs are represented as mean ± SD from experiments repeated in triplicate. *($p < 0.05$), **($p < 0.01$), ***($p < 0.005$) indicate significant difference.

3.4 Treatment-induced morphological changes

Fig. 4 shows morphological changes in MCF-7 cells treated at IC_{50} concentrations. AgNP treatment combined with irradiation (Fig. 4c) induced pronounced morphological changes, including cell rounding, detachment from the culture surface, and membrane damage, compared to the untreated and unirradiated group (Fig. 4a and 4b).

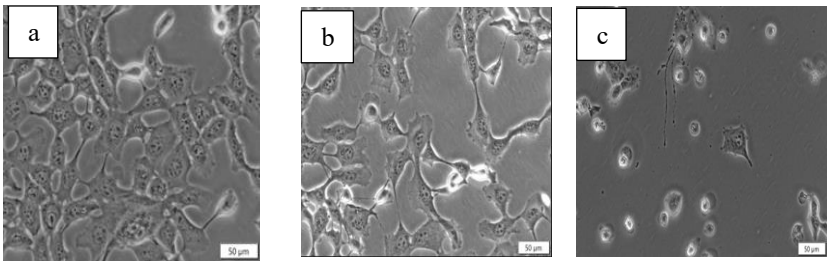

Figure 4. Phase contrast images of MCF-7 cells, (a) untreated control, (b) AgNPs-treated (dark), (c) AgNPs-treated (irradiated) at IC_{50} concentrations under a magnification of 200x.

4 Discussion

The present study explored the chemotoxic and phototoxic potential of chemically synthesized AgNPs against the MCF-7 human breast cancer cell line. UV-Vis spectroscopy and DLS characterization (Fig. 1) confirmed the formation of stable, monodisperse nanoparticles with a characteristic SPR peak at 401 nm and a hydrodynamic size of 119.3 nm, appropriate for cellular interaction and uptake. These physicochemical properties are consistent with literature reports indicating that AgNPs with sizes around 100–120 nm exhibit optimal cellular internalization and interaction for therapeutic purposes [14]. Cell viability analysis demonstrated a dose-dependent cytotoxic response under both dark and irradiated conditions. Notably, the IC_{50} value decreased from 7.3 µg/mL (dark) to 4.3 µg/mL upon 405 nm laser exposure, indicating enhanced cell killing due to phototoxic activation. This enhanced effect is likely driven by the combined influence of ROS generation and localized heat production via the plasmonic properties of AgNPs. These findings align with previous studies demonstrating the synergistic potential of nanoparticle-assisted phototherapy in cancer treatment. El-Hussein and Hamblin similarly observed that light-activated AgNPs induced DNA damage and apoptosis in lung cancer cells through ROS-mediated stress and disruption of mitochondrial membrane potential, indicating that such phototoxic mechanisms may be effective across various cancer cell types [10, 15].

The photothermal analysis confirmed a measurable temperature increase (2.8 °C) in AgNP under 405 nm laser irradiation, compared to a negligible rise (0.9 °C) in the control. While this increase may appear modest, it reflects localized intracellular heating, which, combined with oxidative stress, can initiate apoptosis or necrosis in cancer cells [16]. This is further supported by the observed structural collapse and membrane disruption in treated cells, aligning with reports by Shipunova et al. (2022) where HER2-targeted AgNPs induced potent photothermal apoptosis in cancer models [17]. Morphological observations further supported the cytotoxic effect of AgNPs combined with phototherapy. Cells treated with AgNPs alone exhibited insignificant morphological alterations, compared to the significant structural damage, including membrane disruption and cell collapse in combined treatment group. Thus, our preliminary results suggested, the effectiveness of AgNPs to function as photoresponsive agents that enhance cytotoxicity when activated by blue light.

These findings affirm that citrate-borohydride synthesized AgNPs can induce significant cytotoxicity under laser irradiation, primarily through synergistic ROS generation and local photothermal effects. This supports the growing body of evidence positioning AgNPs as effective dual-mode agents in nanoparticle-mediated phototherapy for breast cancer.

5 Conclusion

This study demonstrated that chemically synthesized AgNPs exhibit enhanced cytotoxic effects against MCF-7 breast cancer cells when irradiated at 405 nm. The ability of AgNPs to generate localized heat and the potential to generate ROS upon irradiation makes them photo-enhanced cancer agents. This offers a promising platform for targeted phototherapy, and further mechanistic investigations will enhance its potential for future *in-vivo* studies.

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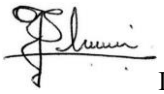
Supervisor Name & Surname: ……..…….. Signature:  Date: ……..…..

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This study presents a novel approach for synthesizing silver nanoparticles using a combined method involving sodium citrate and sodium borohydride. Unlike conventional single-reagent techniques, this approach allows better control over particle size and improves nanoparticle stability. The resulting AgNPs show strong light absorption near 401 nm and respond effectively to 405 nm laser exposure, producing a mild but meaningful temperature increase. This photothermal response, together with the ability to generate reactive oxygen species, enhances their cytotoxic effect on breast cancer cells. The synthesis method directly contributes to the improved therapeutic potential of the metallic plasmonic nanoparticles in phototherapy, combined with its chemotoxicity.

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