CYTOTOXICITY OF *SENNA DIDYMOBOTRYA* LEAVES GREEN-SYNTHESIZED SILVER NANOPARTICLES AND PHOTOTHERAPY AGAINST A375 MELANOMA CELLS

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ABSTRACT

Cancer is a term used to describe a collection of illnesses where the cells within the body undergo unregulated growth and multiplication. It is also worth noting that cancerous cells have the potential of entering the metastatic phase and this allows them of invading adjacent body organs. Currently, there are at least five known conventional treatment modalities for cancer treatment. However, these therapies have been reported of playing a significant role in the induction of tumor recurrence. To address this critical issue, this study anti-proliferative effects of green synthesized silver nanoparticles (AgNPs). The A375 melanoma cell line was employed as the experimental model, and a range of AgNPs concentrations (2, 4, 8, 16 and 32 µg/mL) was applied. Subsequently, the treated cells were subjected to irradiation using a 525 nm diode laser, at a light dose of 10 J/cm², and the morphological changes in cells were observed using a light inverted microscope. Collectively, the results unveiled a dose-dependent reduction in cell proliferation in response to the AgNPs treatment. Taken together, the findings from this study suggests that green-synthesized AgNPs poses significant therapeutic properties as a novel therapeutic approach for the treatment of different forms of cancer, thus offering an effective and eco-friendly therapeutic approach in cancer therapy. The schematic representation of the adopted synthesis and treatment methodology as depicted in the graphical abstract Figure 1.

Graphical abstract

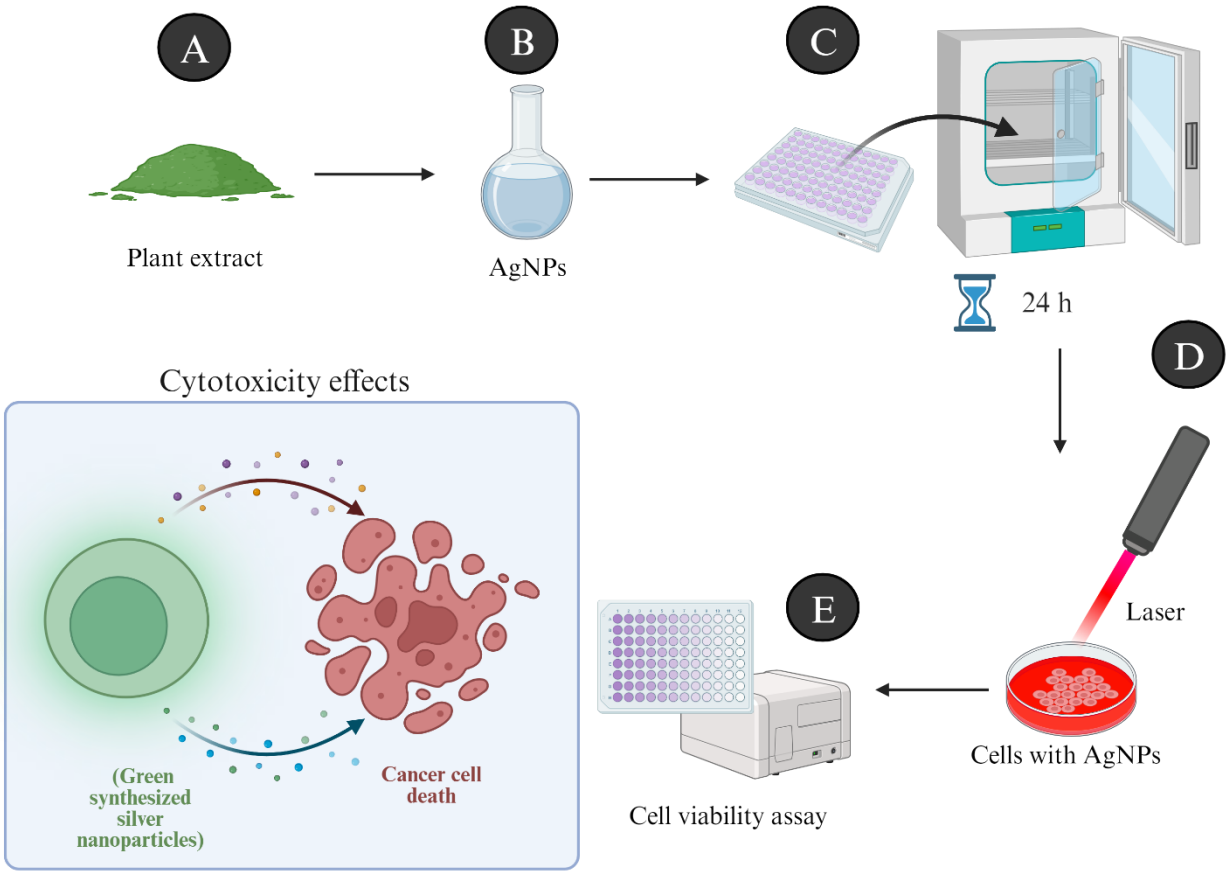


Figure: 1 *A general overview of the methodology adopted for the present study. Ethanol leaf plant extraction using a Soxhlet extractor (A), wet chemistry synthesis of AgNPs using the "bottom-up approach (B), 24 h incubation post-treatment (C), 525 nm laser irradiation (D) and cell viability analysis (E).*

1. INTRODUCTION

Melanoma of the skin presents as a metastatic condition known for its challenging resistance to treatment, and its global incidence has notably surged in recent years. A comprehensive understanding of the initiation, advancement, and spreading of tumors is imperative to address the existing knowledge gaps in melanoma biology. Tumors can manifest on various body surfaces, including the skin, ocular membranes, retroperitoneal space, parenchymatous organs, and various mucosal areas [1]. However, a significant majority, approximately 95%, of all melanoma cases are primarily found on the skin. It's noteworthy that despite their common cellular origin, melanomas should be regarded as a diverse group of diseases rather than a single tumor entity, given their distinct causes, development processes, behaviors, and progression patterns, which can vary based on their location [2]. Despite substantial advancements in comprehending the mechanisms driving melanoma development and progression, there remains a need for deeper mechanistic insights and the exploration of alternative therapeutic strategies [3]. Most importantly, the selection of these treatments depends on the stage and advancement of the tumor. To mitigate some of these adverse effects, numerous researchers are actively investigating fresh therapeutic approaches, such as the integration of photodynamic therapy (PDT) with chemotherapy drugs and phototherapy (PTT). Photodynamic therapy (PDT) represents an emerging treatment modality that employs the use of non-ionizing radiation to induce tumor cell death in different types of cancer [4]. This therapy entails the administration or intravenous delivery of photosensitive drugs, commonly referred to as photosensitizers (PSs), to areas of the body affected by the condition, such as the skin. The fundamental concept underlying PDT relies on the molecular interactions between these photosensitizers, which tend to preferentially accumulate within specific locations of tumor cells, and laser light in conjunction with the presence of molecular oxygen (O_2). This interaction subsequently triggers the production of cytotoxic reactive oxygen species (ROS) [5]. This study explores the cytotoxic effects of eco-friendly green synthesized AgNPs against A375 melanoma cells using a 525 nm diode laser a light dose of 10J/cm^2. Our results demonstrated a clear dose-dependent reduction in cell proliferation. As the concentration of the treatment agent increased, we observed a corresponding decrease in cell growth. Alongside the decrease in cell proliferation, we also noted dose-dependent morphological alterations. These changes in cell appearance were consistent with the reduction in cell growth, suggesting a direct correlation. The dose-dependent reduction in cell proliferation and associated morphological changes suggest that the treatment, involving green synthesized silver nanoparticles and phototherapy, holds promise as a potential therapeutic approach for cancer treatment.

2. METHODS AND METERIALS

2.1 SYNTHESIS OF GREEN SILVER NANOPARTICLES

The plant *Senna didymobotrya* leaves were gathered within the campus premises of the University of KwaZulu-Natal. The plant's leaves were first cleansed and air-dried in the shade. Subsequently, the dried leaf material was finely ground into a powder using a blender. Following this step, the powdered plant material underwent an extraction process using a Soxhlet extractor. This extraction was performed with the aim of synthesizing green silver nanoparticles (AgNPs). To summarize, approximately 10 mL of *Senna didymobotrya* leaf extract stock solution was introduced into a 1 mM AgNO3 solution dissolved in 90 mL of deionized water. This mixture was agitated at a rate of 150 rpm at room temperature. After 6 hours, a noticeable change in the solution's color was observed, indicating a successful reaction. Subsequently, the homogenous mixture was subjected to centrifugation at 12,000 rpm for 10 minutes. The stock solution, which was initially prepared in 0.5% dimethyl sulfoxide (DMSO), was stored at -20°C for future experiments. To confirm the formation of silver nanoparticles (AgNPs), UV-vis is spectroscopy was employed. The UV/Vis analysis covered a spectral range of 300-800 nm and was conducted using a Jenway Genova spectrophotometer.

Figure 2: *Senna Didymobotrya – a visual representation of the plant specimen.*

2.2 CELL CULTURE

A375 melanoma cells were cultivated in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS), 0.1% (v/v) penicillin-streptomycin, and 0.1% (v/v) amphotericin-β. These cells were maintained

at a temperature of 37°C in an environment with 5% CO_2. For in vitro investigations, a seeding density of 3 x 10^5 cells/mL was used for cell plating, and 3.4 cm² culture plates were employed. In this study, two experimental groups were utilized: one comprising cell that did not undergo PtT treatment (referred to as "dark toxicity"), and the other consisting of cells subjected to PTT. Both groups were treated with AgNPs and incubated for 24 hours post-treatment. Various concentrations were applied, encompassing AgNPs (2, 4, 8, 16, and 32 µg/mL) and mediated PTT (2, 4, 8, 16, and 32 µg/mL).

2.3 UV-VIS SPECTROMETRY

Ultraviolet-visible (UV-vis) spectroscopy is a scientific method that utilizes light to investigate the characteristics and behaviors of substances within the UV-vis range of the electromagnetic spectrum. This technique offers valuable insights into phenomena such as electronic transitions in various molecules and their absorption characteristics. To analyze the absorbance spectra of both the plant extract and the silver nanoparticles synthesized through environmentally friendly methods, we employed UV-vis emission spectroscopy. We utilized a UV-Vis Spectrophotometer, specifically the Genova 7315 Life Science Spectrophotometer from JENWAY located at the University of Johannesburg, Laser Research Centre. This instrument was used to measure absorption across the spectral range of 300-800 nm at rtp,

2.4 MORPHOLOGICAL ANALYSIS

Morphological changes were investigated using an Olympus CKX 41 inverted light microscope, which was equipped with an Olympus C5060-ADUS digital camera. The assessment of viable and non-viable A375 cells 24 hours after treatment with AgNPs and AgNPs-mediated PTT. Briefly, cells were subjected to a thrice wash by the using HBSS prior to visualization.

2.5 CELL VIABILITY (ATP LUMINESCENCE ASSAY)

In the current study, we employed the CellTiter-Glo® ATP luminescence assay kit (Promega, G968A). To outline the procedure briefly, approximately 50 µL of a cell suspension was combined with an equal volume of reconstituted ATP reagent. This mixture was thoroughly mixed and allowed to incubate for 10 minutes at room temperature and atmospheric pressure. Following the incubation period, the resulting homogenous colorimetric mixture was then subjected to ATP luminescence measurement using the PerkinElmer VICTOR Nivo™ instrument.

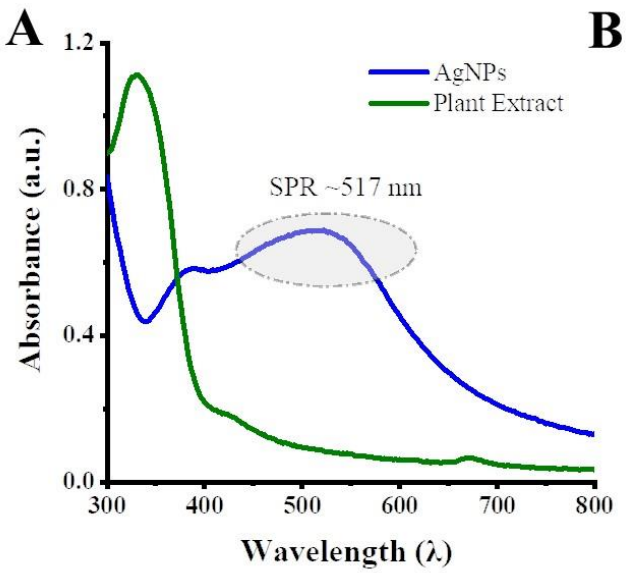
2.6 STATISTICAL ANALYSIS

The experiments were conducted in triplicate (n=3). Data analysis was carried out using IBM SPSS version 27 software to assess the mean differences and determine the statistical significance between the control and experimental groups. The mean values were presented as mean ± standard error (SE), and statistical significance was denoted as follows: $p < 0.001$ (a).

3. RESULTS AND DISCUSSION

3.1 UV-VIS SPECTROMETRY

To investigate the absorbance spectra of both the plant extract and the green synthesized AgNPs by *Senna didymobotrya.* we utilized UV-vis emission spectroscopy. The results obtained from this analysis revealed a prominent surface plasmon resonance peak at approximately 517 nm, as illustrated in Figure 3A, whereas the plant extract has no peak in the therapeutic window of PTT. The UV-vis spectrometry results in our study align closely with those reported by Sytu *et al.*, [7].



B

NAME	PARAMETERS
Drug	AgNPs
Light Source	Diode Laser
Wavelength	525 nm
Wave Emission	Continuous
Spectrum	Blue (Visible)
Fluency	10 J/cm^2

Figure 3: *UV-vis spectrometry of plant extract (green colour) and AgNPs (blue colour) in the spectra range of 300-800 nm (A) and laser parameters (B).*

3.2 MORPHOLOGICAL ANALYSIS

The morphology of both untreated and treated A375 cells is depicted in Figure 4A. After a 24-hour post-treatment period, an analysis of morphological changes in A375 melanoma cells was conducted. When contrasted with the control group, cells subjected to laser light exhibited no significant morphological changes observed. However, when treated cells exposed to dark toxicity and those treated with the 525 nm laser in combination with AgNPs exhibited dose-dependent alterations in cellular morphology. Furthermore, a dose-dependent reduction in ATP proliferation was observed in both the dark toxicity and photothermal therapy (PTT) groups. Particularly, alterations in cell morphology were evident in A375 cells treated with AgNPs and AgNPs-mediated PTT. Even more intriguingly, our findings closely resemble those of another study conducted by Khoza *et al.*, [8].

3.3 CELL VIABILITY (ATP LUMINESCENCEC ASSAY)

The energy levels in both untreated and treated A375 cells were assessed by measuring ATP luminescence levels. In cells subjected to AgNPs-mediated PTT treatment, a dose-dependent reduction in cell proliferation was evident, as depicted in Figure 4B. A simple linear regression analysis demonstrated a negative correlation between relative light units and concentration. This observation strongly indicates that as the concentration increases, the relative light units (%) decrease. However, it's noteworthy that ATP levels were found to be higher in A375 cells that received laser treatment alone. In research conducted by Sriram and colleagues [9], it was demonstrated that silver nanoparticles (AgNPs) possess the ability to differentiate between cancerous and healthy cells. Moreover, they exhibited the capability to trigger anti-tumor effects through the activation of caspase 3, a group of cysteine proteases that play crucial roles in cellular apoptosis pathways. This activation, in turn, resulted in a regenerative response and a subsequent reduction in tumor volume [10]. In a prior study that closely resembles our own research, it was observed that the application of laser irradiation alone or silver nanoparticles (AgNPs) alone did not result in a significant decrease in cell proliferation in the tested cell lines. However, when both laser irradiation and AgNPs were combined in phototherapy (PTT), their synergistic effects led to a notable reduction in cell proliferation within the cancer cell lines [10].

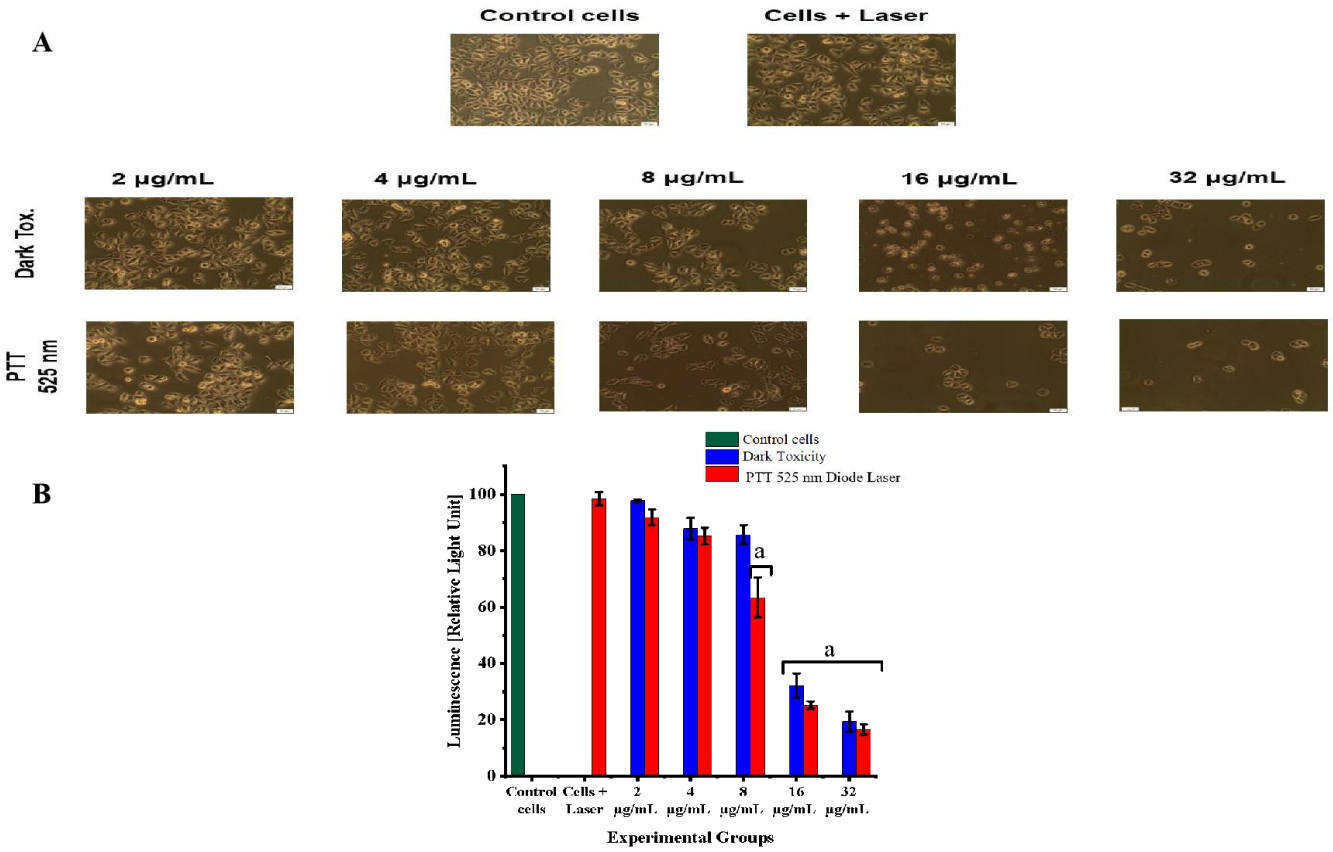


Figure 4: *Morphological (A) and ATP Viability analysis (B) of A375 melanoma cells 24 h post treatment. When compared to control cells, cells treated with laser light did not any significant alterations in morphology. Dark toxicity and 525 nm laser treated cells incubated with AgNPs displayed a dose dependent change in cellular morphology (A) (10X magnification). A dose dependent decrease in ATP viability was observed in both dark toxicity and PTT (B). Significant differences depicted in the graph are denotated as $p < 0.001$(a).*

4. CONCLUSION

In conclusion, our study focused on evaluating the anti-proliferative effects of green synthesized AgNPs in combination with phototherapy against A375 melanoma cells. In our investigation, we used AgNPs at varying concentrations in conjunction with phototherapy using a 525 nm diode laser with a light dose of 10 J/cm². The morphological changes observed through light microscopy demonstrated a dose-dependent reduction in cell proliferation following AgNPs treatment. This promising outcome suggests that green-synthesized AgNPs hold significant therapeutic potential as a novel approach for combating various forms of cancer. Our findings point toward the exciting prospect of utilizing green synthesized AgNPs as an effective and eco-friendly therapeutic strategy in cancer treatment. By leveraging the combination of AgNPs and phototherapy, we may be moving closer to a more efficient and targeted approach to combatting cancer, ultimately offering hope for improved outcomes in cancer therapy. Further research and clinical studies are warranted to validate and expand upon these promising results.

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