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Assessment of Cytotoxicity of Gold Nanoparticles Functionalized With Brazilian Red Propolis in 2D And 3D Models of Urological Cancers

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Extended Abstract

The major urological cancers are prostate adenocarcinoma and urinary bladder [1]. Prostate cancer (PCa) and Bladder cancer (BC) are, respectively, the second and the sixth most frequently diagnosed cancer among men worldwide [2], [3]. Innovative clinical approaches that could result in more effective treatment options, such as gold nanoparticles (AuNPs) functionalized with natural compounds could be offer advantages such as increased efficacy and reduced systemic cytotoxicity [4]. Brazilian red propolis (BRP) is a natural product known for its anti-inflammatory, antioxidant and antitumor properties [5].

In this way, the aims of our research were the development of a therapeutic formulation composed of AuNPs functionalized with BRP and the investigation of their biological activities against urological cancers. Through a green synthesis method, AuNPs were produced using the crude extract of BRP for reducing Au^{+3} to Au^{0}. The dispersion color changed from pale yellow to dark red indicating the production of BRP-AuNPs, which was also confirmed by the formation of a surface plasmon resonance band (SPR band) around 535 nm by UV-VIS Spectrophotometry. Green synthesized AuNPs (BRP-AuNPs) were characterized about hydrodynamic diameter and Polydispersity Index (PDI) by Dynamic Light Scattering (DLS), which demonstrated a monomodal distribution. Zeta Potential value measured by electrophoretic light scattering was negative. Further characterizations were given by Nanoparticle Tracking Analysis (NTA) showing a concentration of nanoparticles of 1,49 $x10^{10}$ particles/mL. Moreover, AuNPs were analyzed by Transmission Electron Microscopy (TEM) showing predominantly spherical morphology and size in the range of 2- 15 nm.

Biological studies were performed to investigate the activity of these nanoparticles against urological cancer cells. Cytotoxicity assays using PC3 (prostate cancer) and RT4 (bladder cancer) cell lines revealed that both BRP extract and BRP-AuNPs exhibited a dose dependent antitumor effect. The IC_{50} values of BRP extract were 58 µg/mL (RT4) and 21.8 µg/mL (PC3), while BRP-AuNPs IC_{50} values were 27.32 µg/mL (RT4) and 53 µg/mL (PC3). These results suggest higher cytotoxic activity of BRP extract when it compared with BRP-AuNPs. Besides that, BRP-AuNPs demonstrated good ability to kill cancer cells. These data were also confirmed by ATP bioluminescence and flow cytometry studies, which indicated a percentage of apoptosis around 44-66%.

Further investigations were performed in urological cancer 3D models. For this, PC3 and RT4 spheroids were treated with BRP extract and nanoparticles dispersion. The cytotoxic activity was investigated by ATP luminescence measurement and also by spheroid images taken before and after the treatment. From these assays, interesting results were acquired, revealing an opposite effect of that demonstrated by 2D viability assays. In 3D viability assays a superior antitumor effect of BRP-AuNPs were exhibited when compared with free BRP extract. These data suggest that nanoparticles are able to penetrate deeper inside the spheroids than the natural compounds, due to their small size. Moreover, live and dead cells in the spheroids were visualized by confocal microscopy after viability dyes staining.

Therefore, our research data have demonstrated that gold nanoparticles were synthesized from Brazilian red propolis by the green synthesis method and also exhibited interesting biological activities against urological cancers, being a potential antitumor formulation.

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