Increased ROS scavenging and antioxidant efficiency of chlorogenic acid compound delivered via a chitosan nanoparticulate system for efficient in vitro visualization and accumulation in human renal adenocarcinoma cells

ABSTRACT

Naturally existing Chlorogenic acid (CGA) is an antioxidant-rich compound reported to act a chemopreventive agent by scavenging free radicals and suppressing cancer-causing mechanisms. Conversely, the compound's poor thermal and pH (neutral and basic) stability, poor solubility, and low cellular permeability have been a huge hindrance for it to exhibit its efficacy as a nutraceutical compound. Supposedly, encapsulation of CGA in chitosan nanoparticles (CNP), nano-sized colloidal delivery vector, could possibly assist in enhancing its antioxidant properties, in vitro cellular accumulation, and increase chemopreventive efficacy at a lower concentration. Hence, in this study, a stable, monodispersed, non-toxic CNP synthesized via ionic gelation method at an optimum parameter (600 µL of 0.5 mg/mL of chitosan and 200 µL of 0.7 mg/mL of tripolyphosphate), denoted as CNP°, was used to encapsulate CGA. Sequence of physicochemical analyses and morphological studies were performed to discern the successful formation of the CNP°-CGA hybrid. Antioxidant property (studied via DPPH (1,1-diphenyl-2-picrylhydrazyl) assay), in vitro antiproliferative activity of CNP°-CGA, and in vitro accumulation of fluorescently labeled (FITC) CNP°-CGA in cancer cells were evaluated. Findings revealed that successful formation of CNP°-CGA hybrid was reveled through an increase in particle size 134.44 ± 18.29 nm (polydispersity index (PDI) 0.29 ± 0.03) as compared to empty CNP°, 80.89 ± 5.16 nm (PDI 0.26 ± 0.01) with a maximal of 12.04 µM CGA loaded per unit weight of CNP° using 20 µM of CGA. This result correlated with Fourier-Transform Infrared (FTIR) spectroscopic analysis, transmission Electron Microscopy (TEM) and field emission scanning (FESEM) electron microscopy, and ImageJ evaluation. The scavenging activity of CNP°-CGA (IC50 5.2 ± 0.10 µM) were conserved and slightly higher than CNP° (IC50 6.4±0.78 µM). An enhanced cellular accumulation of fluorescently labeled CNP°-CGA in the human renal cancer cells (786-O) as early as 30 min and increased time-dependently were observed through fluorescent microscopic visualization and flow cytometric assessment. A significant concentration-dependent antiproliferation activity of encapsulated CGA was achieved at IC50 of 16.20 µM as compared to CGA itself (unable to determine from the cell proliferative assay), implying that the competent delivery vector, chitosan nanoparticle, is able to enhance the intracellular accumulation, antiproliferative activity, and antioxidant properties of CGA at lower concentration as compared to CGA alone.

Keyword: 1,1-Diphenyl-2-picrylhydrazine assay; MTT assay; Chemopreventive; Chitosan nanoparticles; Chlorogenic acid; Drug encapsulated chitosan nanoparticles; In vitro accumulation of encapsulated chlorogenic acid; Nanobiotechnology