World Academy of Science, Engineering and Technology
International Journal of Biomedical and Biological Engineering
Vol:11, No:06, 2017

Azadirachta indica Derived Protein Encapsulated Novel Guar Gum Nanocapsules against Colon Cancer

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Abstract : Azadirachta indica, also known as Neem belonging to the mahogany family is actively gaining interest in the era of modern day medicine due to its extensive applications in homeopathic medicine such as Ayurveda and Unani. More than 140 phytochemicals have been extracted from neem leaves, seed, bark and flowers for agro-medicinal applications. Among the various components, neem leaf protein (NLP) is currently the most investigated active ingredient, due to its immunomodulatory activities against tumor growth. However, these therapeutic ingredients of neem are susceptible to degradation and cannot withstand the drastic pH changes under physiological environment, and therefore, there is an urgent need of an alternative strategy such as a nano-delivery system to exploit its medicinal benefits. This study hypothesizes that guar gum (GG) derived biodegradable nano-carrier based encapsulation of NLP will improve its stability, specificity and sensitivity, thus facilitating targeted anti-cancer therapeutics. GG is a galactomannan derived from the endosperm of the guar beans seeds. Synthesis of guar nanocapsules (NCs) was performed using nanoprecipitation technique where the GG was encapsulated with NLP. Preliminary experiments conducted to characterize the NCs confirmed spherical morphology with a narrow size distribution of 30-40 nm. Differential scanning colorimetric analysis (DSC) validated the stability of these NCs even at a temperature range of 50-60°C which was well within the physiological and storage conditions. Thermogravimetric (TGA) analysis indicated high decomposition temperature of these NCs ranging upto 350°C. Additionally, Fourier Transform Infrared spectroscopy (FTIR) and the SDS-PAGE data acquired confirmed the successful encapsulation of NLP in the NCs. The anti-cancerous therapeutic property of this NC was tested on colon cancer cells (caco-2) as they are one of the most prevalent form of cancer. These NCs (both NLP loaded and void) were also tested on human intestinal epithelial cells (FHs 74) cells to evaluate their effect on normal cells. Cytotoxicity evaluation of the NCs in the cell lines confirmed that the IC50 for NLP in FHs 74 cells was ~2 fold higher than in caco-2 cells, indicating that this nanoformulation system possessed biocompatible anti-cancerous properties Immunoconfocal microscopy analysis confirmed the time dependent internalization of the NCs within 6h. Recent findings performed using Annexin V and PI staining indicated a significant increase ($p \leq 0.001$) in the early and late apoptotic cell population when treated with the NCs signifying the role of NLP in inducing apoptosis in caco-2 cells. This was further validated using Western blot, Polymerase chain reaction (PCR) and Fluorescence activated cell sorter (FACS) aided protein expressional analysis which presented a downregulation of survivin, an anti-apoptotic cell marker and upregulation of Bax/Bcl-2 ratio (pro-apoptotic indicator). Further, both the NLP NC and unencapsulated NLP treatment destabilized the mitochondrial membrane potential subsequently facilitating the release of the pro-apoptotic caspase cascade initiator, cytochrome-c. Future studies will be focused towards granting specificity to these NCs towards cancer cells, along with a comprehensive analysis of the anti-cancer potential of this naturally occurring compound in different cancer and in vivo animal models, will validate the clinical application of this unprecedented protein therapeutic.

Keywords : anti-tumor, guar gum, nanocapsules, neem leaf protein
Conference Title : ICMPNP 2017 : International Conference on Medicinal Plants and Natural Products
Conference Location : San Francisco, United States
Conference Dates : June 07-08, 2017