In vitro and *In vivo* studies of Fisetin and Catechin loaded Calcium phosphate nanoparticles in Breast cancer cell line

Background

Breast cancer, being one of the most occurring cancer in women, accounts for about 14% of cancers in Indian women. Breast cancer affected 2.3 million women globally in 2020, with 685 000 fatalities. Breast cancer had been diagnosed in 7.8 million women in the previous five years as of the end of 2020, making it the most common cancer in the world. According to a recent report published by the National Cancer Registry Program (NCRP), the number of cancer cases is expected to rise by about 20% from 13.9 lakh in 2020 to 15.7 lakh in 2025. Breast cancer is a heterogenous disease and consists of 5 intrinsic subtypes. They are luminal A, luminal B, HER-2 enriched, normal like and triple negative/basal like breast cancers. Doxorubicin, tamoxifen, paclitaxel, 5-fluorouracil, cisplatin, trastuzumab, and bevacizumab are some of the chemotherapy drugs used to treat breast cancer [1]. However, in clinics, serious adverse effects such as heart malfunction limited their treatment approach. Nausea, diarrhea, infection, memory problems, hair loss, and eye problems are some of the other side effects. As a result, there is a need to produce an effective anti-cancer medicine that is nontoxic and has minimum side effects in the treatment of cancer [1]. Natural substances with low toxicity and possible anti-cancer activity were considered for use in the treatment of breast cancer.

MDA-MB-231 is a triple negative breast cancer cell line that accounts for 15% to 20% of all breast cancer cases and is difficult to cure [6]. More research is needed to find new drugs or compounds. According to some studies, fisetin (3,3',4',7-tetrahydroxyflavone) a flavonoid that occurs naturally in fruits and vegetables has anti-cancer properties and induce apoptosis [11]. Catechins, which are present abundantly in green tea leaves (Camellia sinensis), has various health benefits and also shows inhibitory effects on breast cancer [8].

Calcium phosphate nanoparticles (CPNPs) has so many advantages due to their high bioactivity, biocompatibility, chemical stability and strong adsorption ability under physiological conditions [5]. Its average particle size is 15-40nm. They dissolve at a low pH in the surroundings of solid tumors, releasing integrated drugs or biomolecules [2].

This proposal focuses on calcium phosphate nanoparticles as drug carrier for delivering anticancer compounds fisetin and catechin. *In vitro* studies can be performed with MDA-MB-231 cell line to assess toxicity, drug loading and release and apoptosis. In addition, the validation of drug loaded nanoparticles and its associated proteins in *in vivo* model system. Induction of tumor xenograft in nude mice through injection of MDA-MB-231 cells followed reduction of tumor size by injecting our materials and the histopathological changes of tissues can be observed by TEM. Also, the apoptosis through ER and mitochondrial Proteins can be identified.

Objectives

In vitro studies

- Synthesis of CPNP and analyze fisetin and catechin loaded CPNPs.
- Biophysical characterization of CPNPs and fisetin and catechin loaded CPNPs by SEM, TEM, EDX, XRD, FTIR etc.
- CPNP loaded fisetin and catechin treated with MDA-MB-231 cells and assess toxicity, drug loading and release and apoptosis of MDA-MB-231 cells.

In vivo studies

- Induction of tumor in nude mice through injection of MDA-MB-231 cells.
- The different concentrations of fisetin and catechins loaded CPNP injected into the solid tumor and observe the reduction of tumor size.
- Observation of histopathological changes in fisetin and catechin loaded CPNP induces tumor tissues.

Expected outcome

- To get good knowledge about the drug loading into the nano carrier and its various changes and characterization.
- To get detailed analysis about the drug and disease interaction in both *in vitro* and *in vivo* studies.

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