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Novel self-nanoemulsifying formulation of quercetin: Implications of pro-oxidant activity on the anticancer efficacy

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Abstract

The present work focuses on the anticancer potential of quercetin (QT) loaded self-nanoemulsifying drug delivery system (QT-SNEDDS) composed of Capmul MCM, Tween 20 and ethanol. In vitro cell culture studies revealed potential cell cytotoxicity of developed formulation mediated by its ability to induce DNA damage and apoptosis in MCF-7 cells. QT-SNEDDS at a dose of 50 mg/kg demonstrated the antioxidant activity measured as function of prophylactic antitumor efficacy against DMBA induced breast tumors which revealed higher latency to the tumor growth as compared to free QT. This appreciation was further supported by normalized levels of tumor angiogenesis markers (MMP-2, MMP-9, TNF-α and IL-6). At higher doses (100 mg/kg) the pro-oxidant activity was noted and exhibited significantly higher therapeutic anticancer efficacy (~65% tumor suppression) in the same model as compared to that of free QT (~20%). Finally, safety profile of developed formulation was established assessing various hepatotoxicity markers.
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Key words: Quercetin; Prophylactic; Anticancer efficacy; Prooxidant activity; SNEDDS; Apoptosis

Quercetin (QT), a member of polyphenolic flavonoid, is distributed universally in various edible plants and one of the most potent antioxidants of plant origin.[1] It is a safe and commonly ingested dietary flavonoid with varied range of therapeutic benefits including antiviral [2], antidiabetic [3], anti-inflammatory [4] and neuro-protective [5]. Besides being a potential free radical scavenger, it also possesses the concentration dependent pro-oxidant activity[6], which is responsible for its anticancer action on wide variety of cancer and cancer cells.[7-10] Multiple cellular mechanisms at the molecular levels are ascribed to its anti-proliferative action including down regulation of mutant P_{53} protein [11], G1 phase arrest [12], inhibition of tyrosine kinase [13] and estrogen receptor binding.[14] Additionally, QT also has potential anti-angiogenic propensity[15], owing to its inhibitory action upon vascular endothelial growth factor receptors (VEGFR) [16], matrix metalloproteinase (MMP-9), and cyclooxygenase (COX-2).[17]

Despite the potential application of QT in cancer therapy, its clinical use is limited because of its poor biopharmaceutical properties, which raise the need of high oral dose (250 to 500 mg *tid*) to be administered. Although it possesses fair intestinal permeability, it suffers from poor aqueous solubility (10 μg/ml).[18,19] Furthermore, orally administered QT is extensively metabolized before its entry into the blood and internal organs suggesting its biotransformation in gastrointestinal tract (GIT) and/or liver.[20] All these factors cumulatively hamper the oral bioavailability of QT (rats < 17%; humans < 1%), thereby limiting its therapeutic efficacy by the conventional dosage form.[21,22]

Recently, in our previous report we have developed self-nanoemulsifying drug delivery system (SNEDDS) of QT for oral bioavailability enhancement. Exhaustive optimization was carried out to have a formulation comprising of GRAS listed

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excipients within their IIG limits. In vivo pharmacokinetics revealed ~5-fold increase in the oral bioavailability of QT when loaded in SNEDDS. Additionally, the formulation also showed higher antioxidant potential as compared to free QT, measured as a function of its ability to combat the drugs (Cyclosporin A and Doxorubicin) induced toxicity.[23]

Although various nanoformulations have been designed to improve the anticancer potential of QT, most of the studies are limited only to in vitro testing on cancer cell lines.[24-29] Further, potential of nanocarriers in improving the anticancer efficacy of QT following oral administration is least explored till date. In this regard, Date and co-workers reported the potential of orally administered phospholipid-based cationic nanocarriers in improving the antitumor efficacy of QT in C57BL/6 mice.[30] On the similar line of action, Tan et al demonstrated the augmented anticancer activity of QT in nano-miceller formulation following oral administration to murine xenograft based animal model.[31] Nevertheless, the efficient therapeutic application of the aforementioned delivery system is limited by poor loading of QT and poor industrial scalability. Furthermore, the miceller system may lose its structural architecture in differential pH of GIT.

Herein, we stemmed our work to investigate the anticancer potential of previously developed QT-SNEDDS.[23] Both prophylactic and therapeutic anticancer efficacy of the formulation was evaluated in the 7,12-dimethylbenz[α] anthracene (DMBA) induced tumor bearing animals. A meaningful correlation of the in vivo antitumor efficacy of QT-SNEDDS was carried out with in vitro cytotoxicity, DNA damage and apoptotic activity against MCF-7 cells.

Materials and methods

Materials

QT (anhydrous), 7,12-dimethylbenz[α] anthracene (DMBA), 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Triton X-100, 4′,6-diamidino-2-phenylindole (DAPI) and Coumarin-6 (C-6) were purchased form Sigma, USA. Capmul® MCM was procured as generous gift from Abitech Corporation, Janesville, USA. Ultra-pure deionized water (LaboStar™ ultrapure water Systems, Germany) was used for all the experiments. All other reagents used were of analytical grade.

Preparation and characterization of QT loaded SNEDDS (QT-SNEDDS)

QT-SNEDDS was prepared by using Capmul MCM, Tween 20 and ethanol as oil, surfactant and co-surfactant, respectively.[23] Briefly, excess amount of QT was added to glass vial containing the mixture of Capmul MCM (400 mg), Tween 20 (400 mg) and ethanol (200 mg), followed by vortexing for 2 min to obtain a homogenous mixture. The resultant mixture was allowed to incubate in shaker water bath (Lab Tech, Korea) operated at 50 strokes/min for 72 h at 37 °C to attain the equilibrium. The mixture was centrifuged at 5000 rpm for 5 min to separate insoluble drugs followed by heating of supernatant at 40-45 °C to form the QT-SNEDDS. The formulation (200 mg) was diluted with 50 ml of simulated gastric fluid (SGF; pH 1.2) to form the nanoemulsion, which was allowed to stand for 2 h to attain the equilibrium. Various formulation attributes of nanoemulsion such as droplet size and PDI were evaluated using Zeta Sizer (Malvern Instrument, UK).[23]

Cell culture experiments

Cell culture

In vitro anticancer efficacy of QT-SNEDDS was evaluated in Human breast adenocarcinoma cells (MCF-7; American Type Culture Collection (ATCC) Manassas, VA, USA). MCF-7 cells were grown in Minimum Essential Medium Eagle (MEM, Sigma) supplemented with Earle's salts, L-glutamine, non-essential amino acids, sodium bicarbonate, sodium pyruvate, 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin (PAA Laboratories GmbH, Austria) at 37 °C in 5% CO_2 and 95% humidified atmosphere. The cell culture medium was changed at every alternate day. Confluent cultures were harvested by trypsinization with 0.25% trypsin-EDTA solution (Sigma) and cells were further employed for cell uptake, cell cytotoxicity, DNA damage and apoptosis studies.

Cell uptake and intracellular localization studies

Qualitative uptake analysis of QT-SNEDDS in MCF-7 cells was carried out by confocal laser scanning microscope (CLSM). Cells were seeded in 6 well culture plate (50,000 cells/well) (Costars, Corning Inc., NY, USA) and allowed to incubate overnight for cell attachment. For intracellular localization studies, fluorescent QT-SNEDDS (C-6-QT-SNEDDS) was prepared by solubilizing C-6 (1 mg) in QT-SNEDDS. C-6-QT-SNEDDS was suitably diluted with cell culture medium and allowed to stand for 15 min to attain the equilibrium. MCF-7 cells were incubated with diluted QT-SNEDDS (equivalent 1 μg/ml to free C-6) for 1 h followed by fixing with 3% paraformaldehyde (Merck, India) and permeabilized with 0.2% Triton X-100. The nuclei of the cells were stained with 10 μg/ml DAPI (Sigma, USA) and finally cells were observed under the CLSM (Olympus FV1000).

Quantitative cell uptake

Time and concentration dependent cell uptake of QT from QT-SNEDDS was evaluated in MCF-7 cells, which were seeded at a density of 1,00,000 cells/well in 24 well cell culture plates (Costars, Corning Inc., NY, USA) and allowed to attach overnight. Evaluation of concentration dependent cell uptake was carried out by incubating the MCF-7 cells with fresh medium containing varying concentration of free QT and QT-SNEDDS and further incubated for 1 h. Likewise, cell uptake of QT was also evaluated in time dependent manner by incubating appropriate concentration of free QT and QT-SNEDDS for varying time intervals (0.5, 1, 1.5, 2 h). Further, cells were lysed with 0.1% w/v Triton X-100 followed by extraction with methanol to completely solubilize the internalized drug. The cell lysate was centrifuged at 21,000 rpm for 10 min and obtained supernatant was subjected to HPLC analysis for quantification of internalized drugs.[23]

Cell cytotoxicity assay

Following the attachment of cells (10,000 cells/ well) in 96 well cell culture plates (Costars, Corning Inc., NY, USA),

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