Now-a-days plant based phytochemicals are gaining much importance over conventional therapeutics owing to their multifunctionality and low toxicity. Thymoquinone (TQ), is a bioactive molecule isolated from the volatile oil fraction of *Nigella sativa*, is one such phytochemicals which possess remarkable anti-cancer activity and known for its anti-inflammatory, anti-oxidant, anti-fungal and anti-bacterial activity. Studies with TQ has shown its potential as an antibacterial agent and in the present study, this ability has been explored in detail. Antibacterial activity of TQ was studied by determining minimum inhibitory concentration, minimum bactericidal concentration, time-kill assay and post-antibiotic effect against two Gram-negative and two Gram-positive bacteria. The minimum inhibitory concentration of TQ was found to be in the range of 1.56 µg/ml to 100 µg/ml. Treated bacterial cells at MIC were analyzed using scanning electron microscopy which revealed changes in cell morphology, cell lysis and cell size reduction. Live/dead imaging confirmed the bactericidal activity of TQ as treated bacteria showed uptake of ethidium bromide over acridine orange. Selectivity of TQ towards bacterial cell was found by studying its toxicity towards HaCaT (human keratinocytes) cell line by MTT assay and IC$_{90}$ value was found to be 50µg/ml which was higher than that of MIC$_{bacteria}$ (except for MIC of *E. coli*). TQ also showed promising anti-biofilm activity against Gram-negative (*E. coli* and *P. aeruginosa*) and Gram-positive bacteria (*B. subtilis* and *S. aureus*), which was studied by crystal violet assay, MTT assay, CFU counting and SEM. TQ also exhibited anti-biofilm activity against mature biofilm i.e. 6 h old and 24 h old. For understanding the antibacterial mechanism of action of TQ, DiSC3, NPN and ROS assay was performed. DiSC3 and NPN assay has not shown any membrane damage caused by TQ. However, bacterial cells treated with TQ at MIC showed increased
dichlorofluorescin fluorescence suggesting the production of reactive oxygen species. This was further confirmed by incubating bacteria with MIC of TQ in presence of reduced glutathione, a known ROS scavenger. Glutathione caused attenuation of TQ’s antibacterial activity confirming that ROS generation could be the probable mechanism for TQ antibacterial agent.

In this study, nanoencapsulation of TQ within PLGA and mesoporous silica nanoparticle was carried out, to overcome the limitations of using TQ in its free form. PLGA was employed as a nanocarrier because of its biocompatibility, biodegradability and property of sustained release of drug over long period of time. Iron-oxide nanoparticles of size 6-10 nm has also been incorporated within PLGA along with TQ to impart the benefit of targeted drug delivery and also modulation of drug release kinetics can be achieved by hyperthermia. Advantages with mesoporous silica nanoparticles are non-toxicity, high surface area to volume ratio, high porosity and chemical stability which leads to high loading efficiency of hydrophobic drugs.

Successful encapsulation of TQ within PLGA and mesoporous silica nanoparticles has been achieved with size in the range of 100 nm- 300 nm. Both TQ nano-formulations showed cytotoxicity against HeLa and MCF-7 cell lines at lower concentration of TQ as compared to the free TQ. Anticancer activity of TQ loaded PLGA and MSNPs were studied by DAPI staining and Annexin V-FITC/PI staining and it was found that both nanoformulations exhibit anticancer activity by inducing apoptosis.