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Thymoquinone inhibits biofilm formation and has selective antibacterial activity due to ROS generation

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Abstract

The present study was aimed to investigate the antibacterial potential and antibiofilm activity of thymoquinone and its mechanism of action. Antibacterial activity of thymoquinone was studied using minimum inhibitory concentration, minimum bactericidal concentration, time-kill assay, and post-antibiotic effect. Thymoquinone exhibited antibacterial activity against both Gram-negative and Gram-positive bacteria. In this study, the minimum inhibitory concentration was found to be in the range of 1.56 to 100 µg/ml. Scanning electron microscopy imaging revealed changes in cell morphology with dents, cell lysis, and reduction in cell size. Live/dead imaging using acridine orange and ethidium bromide confirmed the bactericidal activity as treated bacteria showed selective uptake of ethidium bromide over acridine orange. Cell viability was also studied using HaCaT (human keratinocytes) cell line by MTT assay, and IC₉₀ value was found to be 50 µg/ml. This IC₉₀ value was higher than that of MIC_{bacteria} (except for MIC of *E. coli*), demonstrating that its selectivity is higher towards bacteria than normal human cells. Thymoquinone also showed promising antibiofilm 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, CFU method, and SEM. For understanding the mechanism of action of thymoquinone, DiSC3, NPN, and ROS assay was performed. DiSC3 and NPN assay has not shown any membrane damage whereas bacterial cells treated with thymoquinone at MIC showed increased dichlorofluorescein fluorescence, suggesting that the probable mechanism of action of thymoquinone against bacterial cells is due to the production of reactive oxygen species.

Keywords: Antibacterial; Antibiofilm; Mechanism of action; Reactive oxygen species; Thymoquinone.

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