Title:	Gentamicin Augments the Quorum Quenching Potential of Cinnamaldehyde In Vitro and Protects Caenorhabditis elegans From Pseudomonas aeruginosa Infection
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Abstract:	The quorum sensing (QS) circuitry of Pseudomonas aeruginosa represents an attractive target to attenuate bacterial virulence and antibiotic resistance. In this context, phytochemicals harboring anti-virulent properties have emerged as an alternative medicine to combat pseudomonal infections. Hence, this study was undertaken to investigate the synergistic effects and quorum quenching (QQ) potential of cinnamaldehyde (CiNN) in combination with gentamicin (GeN) against P. aeruginosa. The QQ activity of this novel combination was evaluated using a QS reporter strain and synergism was studied using chequerboard assays. Further, the genotypic and phenotypic expression of pseudomonal virulence factors was examined alongside biofilm formation. The combination of CiNN and GeN exhibited synergy and promising anti-QS activity. This drug combination was shown to suppress AHL production and downregulate the expression of critical QS genes in P. aeruginosa PAO1. Molecular docking revealed strong interactions between the QS receptors and CiNN, asserting its QQ potential. Bacterial motility was compromised along with a significant reduction in pyocyanin (72.3%), alginate (58.7%), rhamnolipid (33.6%), hemolysin (82.6%), protease (70.9%), and elastase (63.9%) production. The drug combination successfully eradicated preformed biofilms and inhibited biofilm formation by abrogating EPS production. Our findings suggest that although GeN alone could not attenuate QS, but was able to augment the anti-QS potential of CiNN. To validate our results using an infection model, we quantified the survival rates of Caenorhabditis elegans following PAO1 challenge. The combination significantly rescued C. elegans from PAO1 infection and improved its survival rate by 54% at 96 h. In summary, this study is the first to elucidate the mechanism behind the QQ prospects of CiNN (augmented in presence of GeN) by abrogating AHL production and increasing the survival rate of C. elegans, thereby highlighting its anti-virulent properties.
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