ABSTRACT

Cantharidin (CAN) is a terpenoid compound of insect origin with the Spanish fly, *Cantharis vesicatoria*, probably being the best-known source. CAN is naturally produced by the male blister beetles as an anti-predatory mechanism and is presented to their female partners as a copulatory gift to defend their eggs from predators. It was initially discovered in 1910 by a French chemist Robiquet. Since the time of its discovery, this molecule has been widely studied to understand its mechanism(s) of action and its role as an anticancer molecule. So far CAN has been shown to induce DNA damage, cell death through G2/M phase cell cycle arrest, apoptosis and MAPK signaling pathway Additionally, it is known to suppress protein phosphatase 2A (PP2A), a positive regulator of cell growth and division, and cancer metastasis. In yeast, CAN has been reported to be rendered inactive post methylation by a protein cantharidin resistance gene 1 (Crg1). Recent findings suggest that CAN exerts its toxic effects by perturbing lipid homeostasis and targeting Cdc1 mediated remodelling in yeast ER. Although we have some knowledge about its mechanism of action, its other potential genetic/epigenetic targets remain to be explored. Also, CAN associated drug resistance mechanisms which dictates the cytotoxic potential of this drug is yet to be identified.

Through our present study, we have identified new molecular mechanism(s) behind cantharidin mediated cytotoxicity using *Saccharomyces cerevisiae* as a model organism. Our findings suggest yeast cells are sensitive to CAN and external supplementation of ethanolamine (ETA) could ameliorate this cytotoxicity. Further, cantharidin downregulates phosphatidylserine decarboxylase1(*PSD1*) expression which in turn affects the cellular PE pool. We show cantharidin inhibits autophagic flux and external administration of ETA could rescue this inhibition. Next, we investigated CAN associated drug resistance mechanisms which dictates the efficacy and cytotoxic potential of this drug. To this end, we screened mutants of pleiotropic drug resistance network of genes for their susceptibility to CAN. Loss of one of the Pdr1 target genes, *PDR5*, encoding an ABC membrane efflux pump, rendered the cells hypersensitive whereas overexpression of it conferred resistance. We also explored the role of histone H3/H4 residues in mediating CAN toxicity. Interestingly, mutations in several histone residues caused drastic reductions in CAN drug resistance

and *PDR5* transcription. Collectively, our findings reveal that CAN mediated cytotoxicity in yeast occurs via downregulation of *PSD1* expression and defects in autophagic flux and Pdr5 serves as a major safeguard against this cytotoxicity.

Keywords: Cantharidin, *PSD1*, autophagy, stress response, yeast, Kennedy pathway, ABC transporter, *PDR1*, *PDR3*, histone, gene expression, histone H3/H4, transcription regulation, cytotoxicity, Chloroquine