ESM 2:

Effects of Piperine in an in vitro model of DTT-induced ER Stress in NRK-52E cells

Hammad et al. 2017

DTT-induced ER Stress Model: Following 24 h pretreatment of NRK-52E cells with Piperine (250 and 500 nM), cells were exposed to 5 mM DTT for 5 hours. Cells were then washed and replenished with fresh media and incubated for 19 hours in 37^{o}C. At the endpoint, cell viability was assessed using Alamar blue assay, and the cell lysates were collected for western blotting to probe for ER stress markers – GRP78 and CHOP.

GRP78 Expression

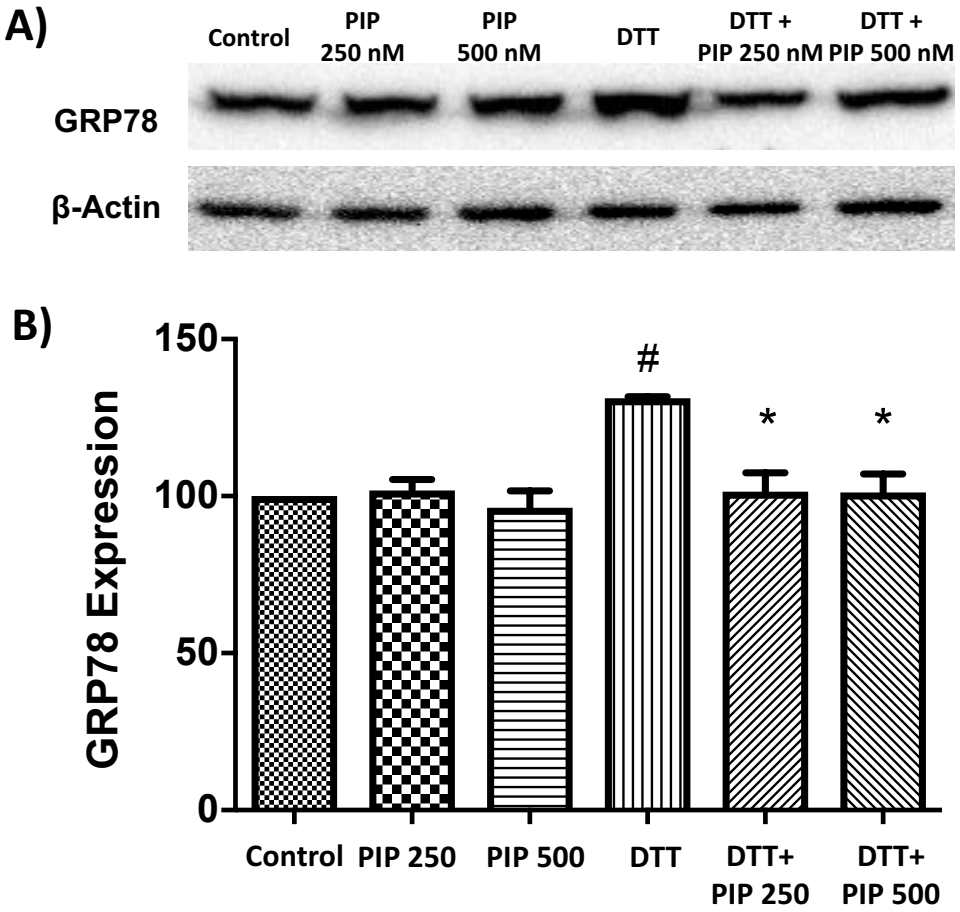

Figure 1: *Effect of Piperine on DTT-induced expression of GRP78 in NRK-52E cells as determined by western blotting (**A**) and quantified using densitometry (**B**). Values were normalized using β-actin and expressed as percentage of vehicle-treated control (Mean ± SEM; n = 3). #P< 0.05 compared to Vehicle-treated control group; *P < 0.05 compared to DTT-treated group*

CHOP Expression

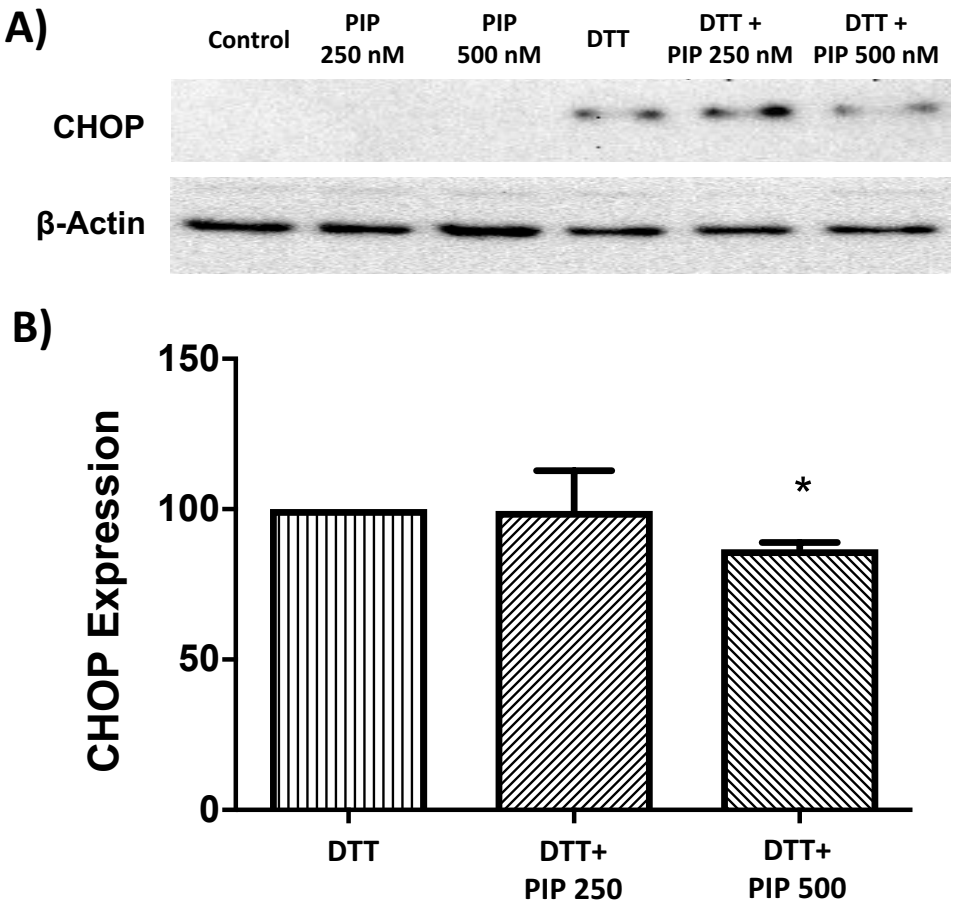

Figure 2: *Effect of Piperine on DTT-induced expression of CHOP in NRK-52E cells as determined by western blotting (**A**) and quantified using densitometry (**B**). Values were normalized using β-actin and expressed as percentage of DTT-treated group (Mean ± SEM; n = 3). *P < 0.05 compared to DTT-treated group*

Cell Viability - Alamar blue assay

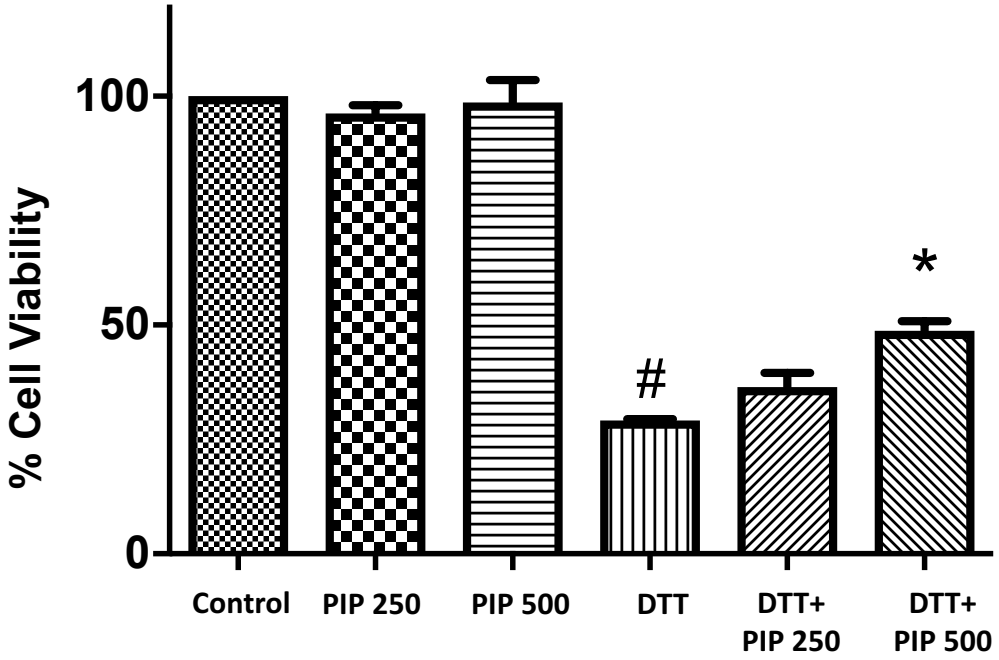

Figure 3: *Effect of Piperine on DTT-induced loss of cell viability (measured by Alamar blue assay) in NRK-52E cells. Fluorescence values were normalized to vehicle-treated control and expressed as Mean ± SEM (n = 4). #P< 0.05 compared to vehicle-treated control group.*