Melatonin induced suppression of ER stress and mitochondrial dysfunction inhibited NLRP3 inflammasome activation in COPD mice

Sushweta Mahalanobish , Sayanta Dutta , Sukanya Saha , Parames C. Sil *

Division of Molecular Medicine, Bose Institute, P-1/12, CIT Scheme VII M, Kolkata, 700054, India

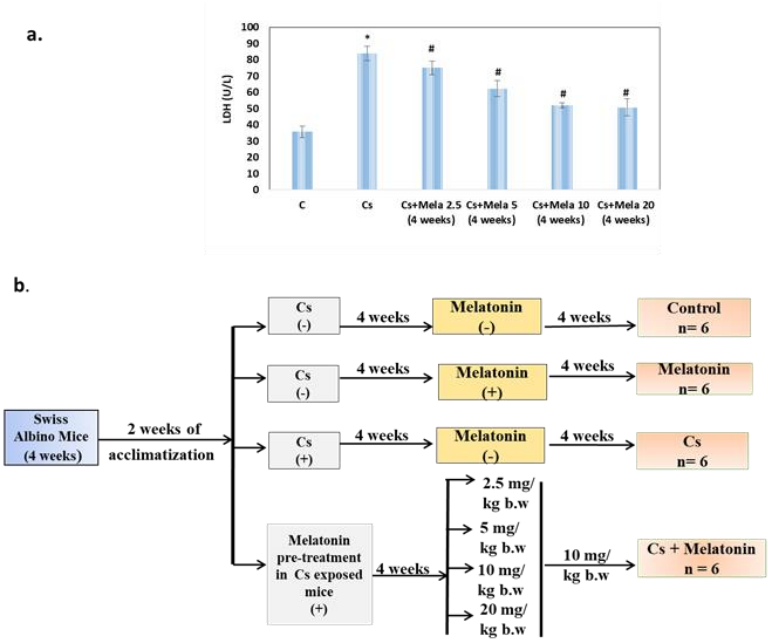
doi: https://doi.org/10.1016/j.fct.2020.111588

Chronic obstructive pulmonary disease (COPD) is a chronic heterogeneous inflammatory disease characterized by airway remodelling and reduced airflow. In recent decades, COPD occurrence is increased considerably among young population that ultimately end-up into deadly lung cancer. COPD triggers the inflammation in airway that causes inflammosome mediated cell death. In inflammosomal pathway, activation of NLRP3 sensors Melatonin is a pineal gland derived hormone that shows effective free radical scavenging property to upregulates the cellular antioxidant potential. But its role in inflammasome mitigation in COPD state is not explored. Therefore in our study we investigated immunomodulatory role of melatonin against COPD induced pyroptosis.

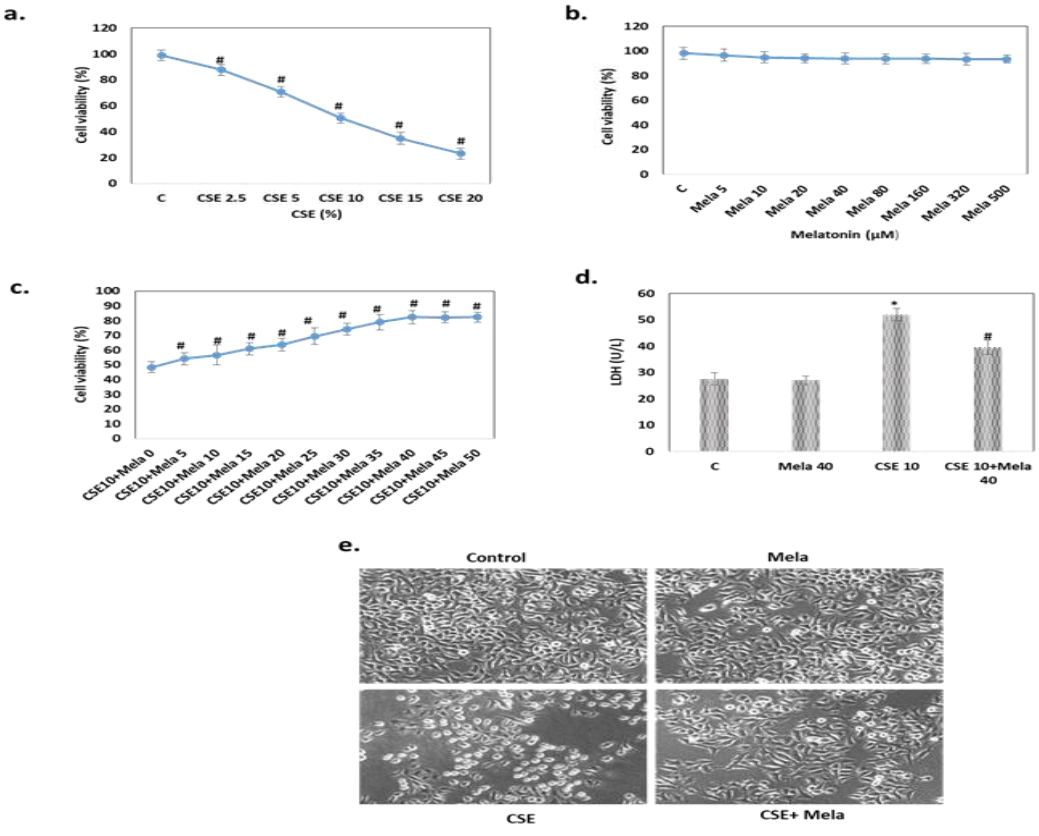
In our study, we developed COPD mice model and cigarette smoke extract (CSE)-treated in vitro L-132 (alveolar epithelial cell) model. Animals exposed to cigarette smoke (Cs) (4hr/day upto 4 weeks) developed increased production of broncho alveolar lavage fluid (BALF) lactate dehydrogenase level and alveolar structure disruption. There were exaggerated production of reactive oxygen species (ROS), deficiency of antioxidant status with altered GSH/GSSG production due to Cs exposure. Cs induced ER stress by upregulating ER stress sensors IRE1-α, PERK, eIF2-α that trigger the dissociation of TXNIP from TRX-1 and NLRP3 inflammasome activation that induce IL-1β, IL-18 mediated pyroptotic cell death. Cs also triggered excessive mitochondrial ROS generation and mitochondrial health impairment (disruption of mitochondrial membrane potential, reduced ATP production). Excessive accumulation of Ca^{+2} in ER caused the diffusion of Ca^{+2} from ER to mitochondria via opening of mitochondrial permeability transition pore by VDAC-1 upregulation. Excessive production of mt.ROS also trigger NLRP3 mediated cell death. Melatonin administration restored the antioxidant defence mechanism by upregulating the expression of NRF-2. Lung inflammation after melatonin intake was significantly reduced due to TXNIP upregulation. Moreover increased clearance of impaired mitochondria by upregulating PINK-1, Parkin, LC3B-II expression reduced mitochondria mediated NLRP3 inflammasome formation. Melatonin therefore suppressed ER stress and mitochondrial dysfunction mediated NLRP3 formation in COPD mice.

Results:

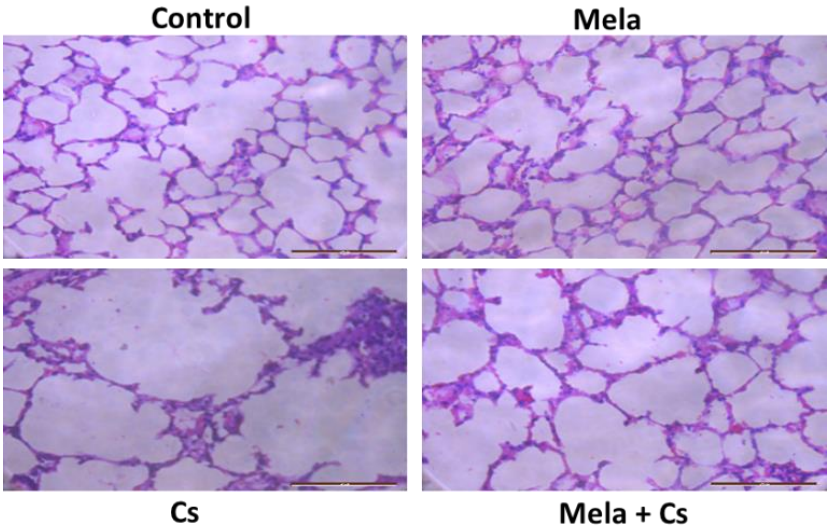
1. Dose dependent assay and schematic representation of the protocol



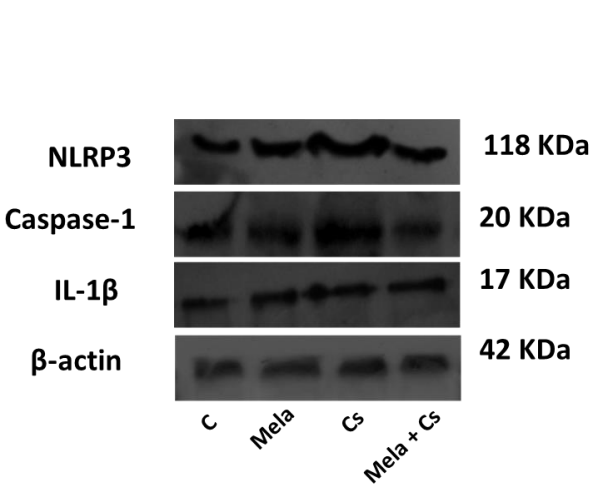
2. Dose dependent study in L-132 cell line

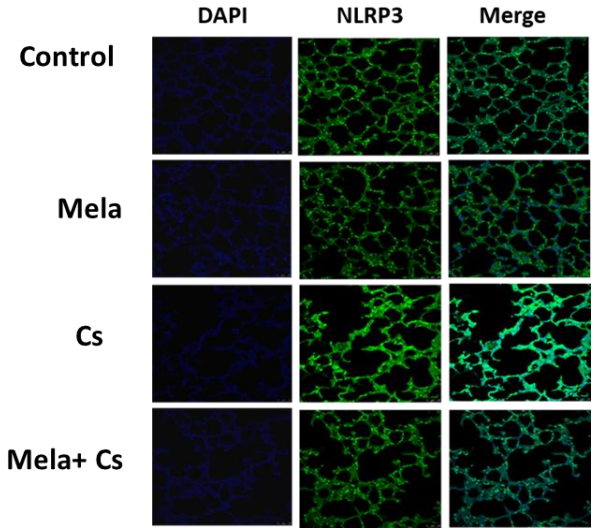


2. Histological alteration:

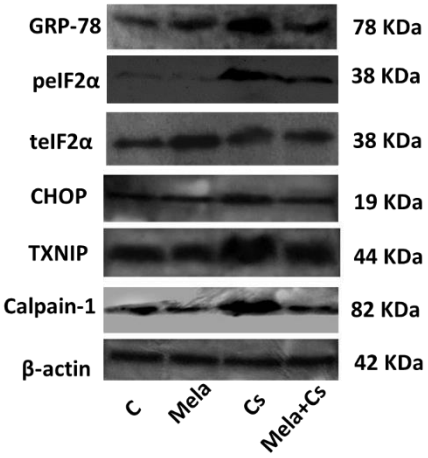


3. Activation of inflammasomal pathway due to Cs exposure

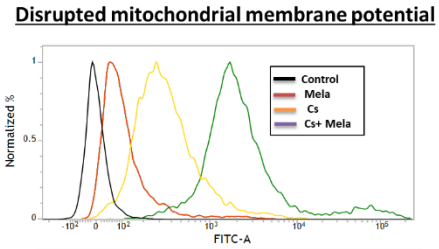


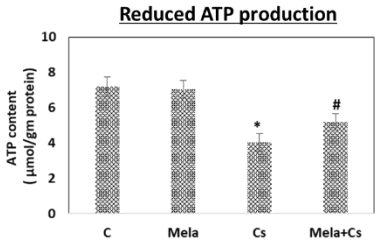


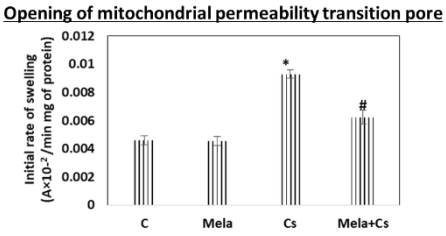
4. Induction of ER stress

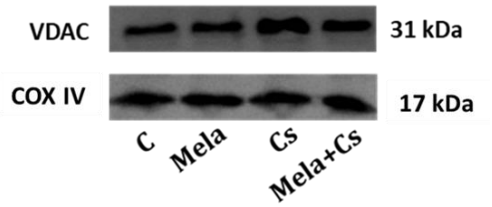


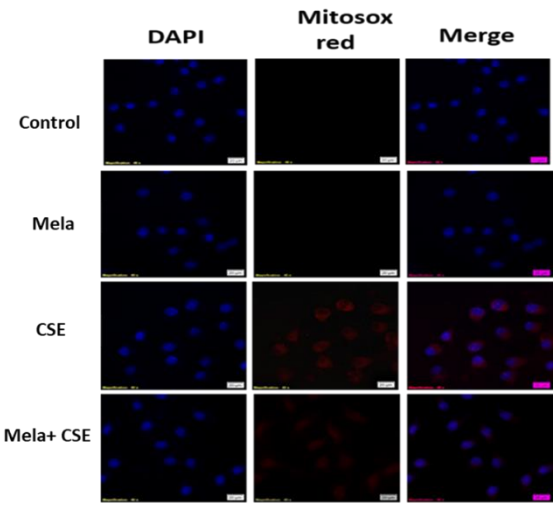
5. Induction of mitochondrial ROS and mitochondrial damage



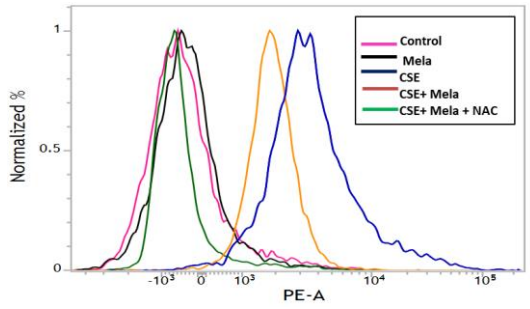


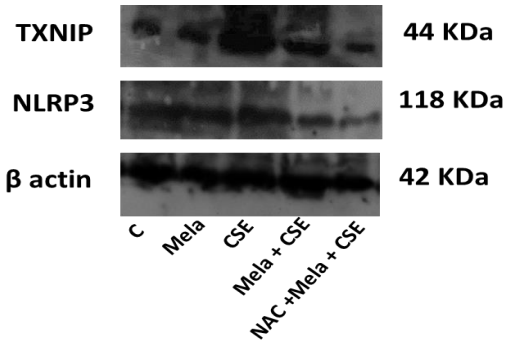






6. Inhibition of ROS suppressed TXNIP and mitochondrial ROS mediated inflammasome formation





7. Upregulation of antioxidant gene expression and mitophagy by melatonin