PONDICHERRY CENTRE FOR BIOLOGICAL SCIENCE AND EDUCATIONAL TRUST

Reg.No.2840/B4/2016 Pondicherry

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SUSTAINABLE RESEARCH FOR BETTERMENT OF MANKIND

CERTIFICATE OF ANALYSIS

PCBSET/2022-2023/RN004	Date: 12/04/2022

User information: **Mrs. J. Manonmani,**
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India

Sample information: Powder

Sample Code: G.A, S.A, C.A

Analysis: Intracellular ROS analysis using DCFDA staining

Date of sample received: 30/01/2022

Date of Analysis: 29/03/2022

Date of report: 12/04/2022

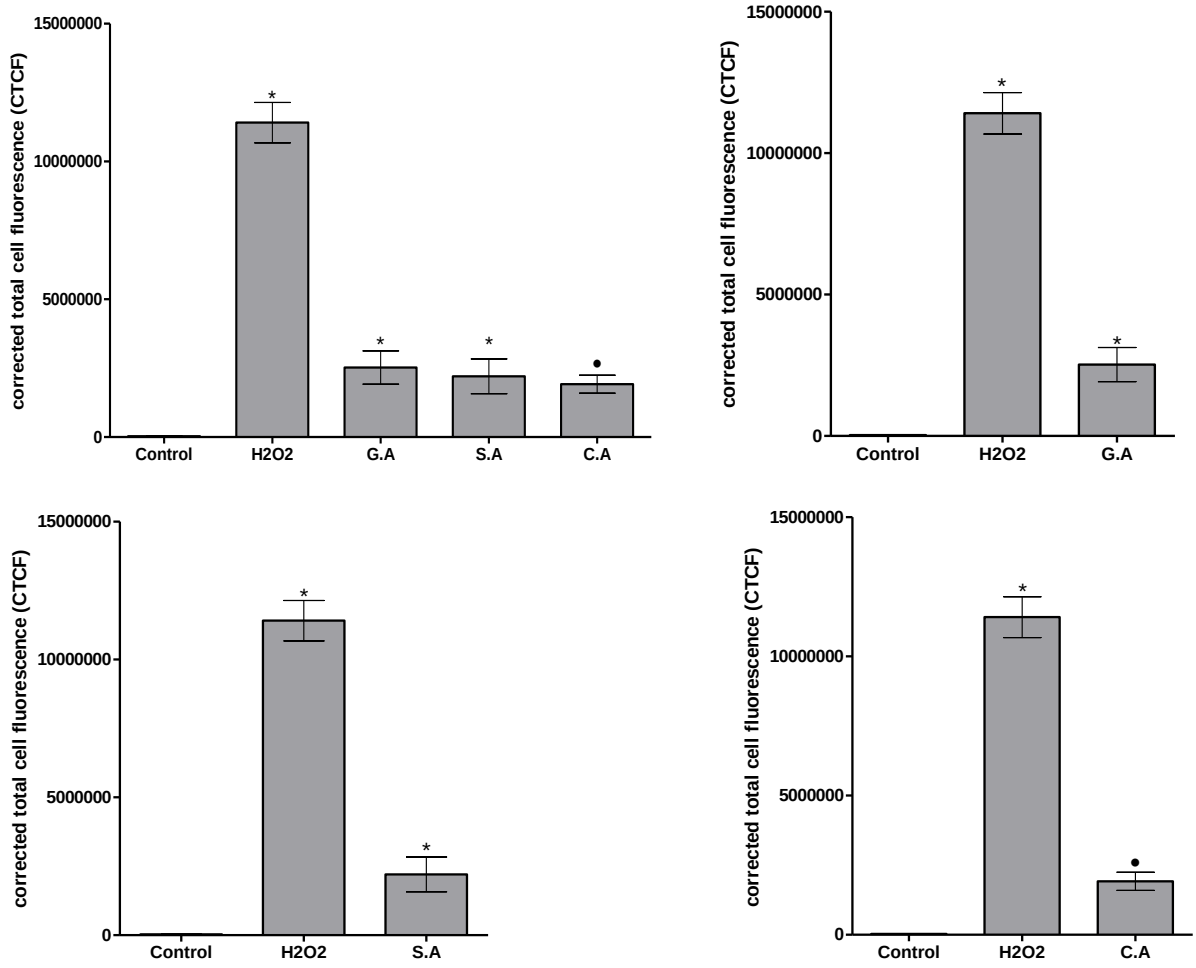
Methodology:

ROS measurement was performed using H_2DCFDA as described by Ling et al., 2011. In brief, each 12-well plates were seeded with 2 X 10^5 cells per well and allowed to adhere overnight. The test material was added and incubated for 8 h. H_2O_2 was used as a positive control. Following the drug treatment, media was removed and cells were loaded with 5 µM H_2DCFDA diluted in clear media for 30 min at 37 ˚C. Cells were washed three times with sterile PBS and morphology of cells was photographed using OPTIKA INVERTED MICROSCOPE-IM Series (Optika Srl.,Italy) and the florescent images were captured under green filter. For quantification, the average fluorescence of the total population was measured by ImageJ software and a histogram was plotted with the relative fluorescence from six independent sets of data. The fluorescence intensity was calculated using the calculation for corrected total cell fluorescence (CTCF) = integrated density–(area of selected cell × mean fluorescence of background readings), as described by Jakic et al (2017).

Reference:

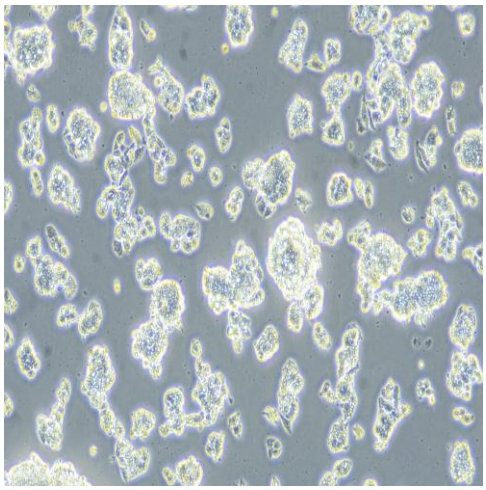
1. Ling LU, Tan KB, Lin H and Chiu GNC (2011). The role of reactive oxygen species and autophagy in safingol-induced cell death. Cell Death and Disease (2011) 2, e129; doi:10.1038/cddis.2011.12.
2. Jakic B, Buszko M, Cappellano G, Wick G. Elevated sodium leads to the increased expression of HSP60 and induces apoptosis in HUVECs. PLoS One. 2017;12(6):e0179383–3.

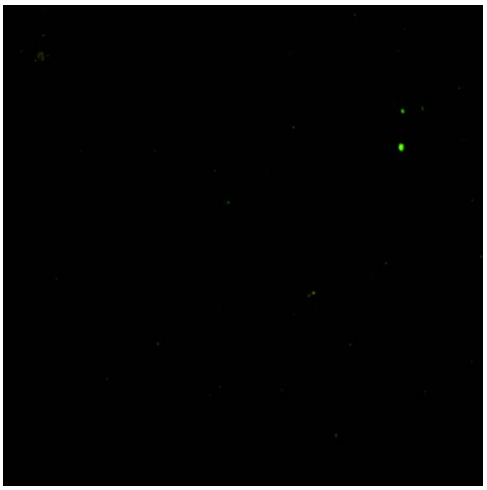
Results:



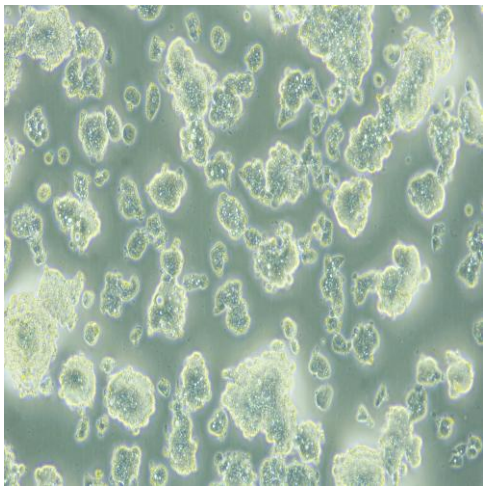
Detection of ROS generation by DCFDA. G.A & S.A showed an increase in ROS generation compared to C.A as revealed by increase in cell fluorescence (* denotes $p < 0.01$; • denotes $p < 0.1$). The histogram was prepared with a mean fluorescence of the control and treated group.

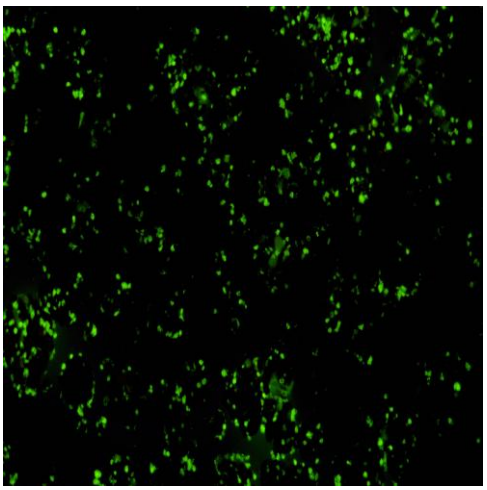
Control



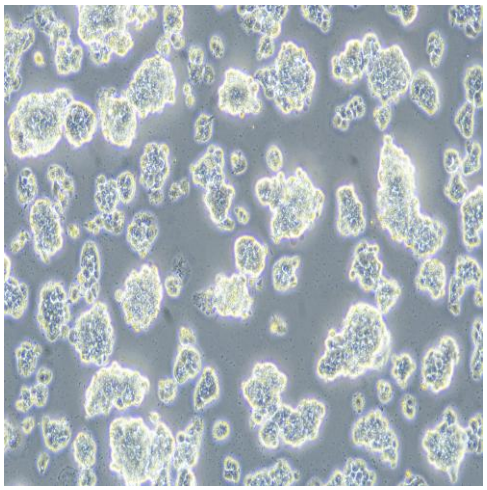


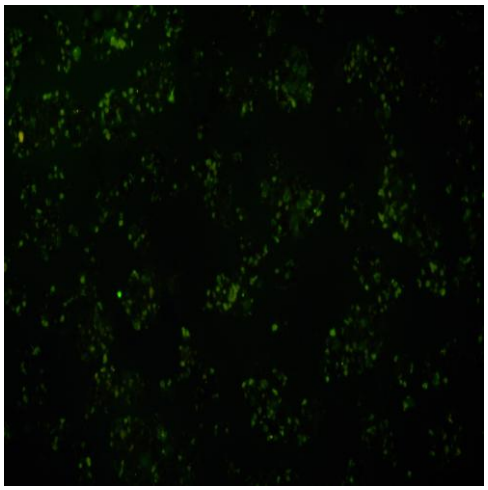
Positive Control (200µM H_2O_2)



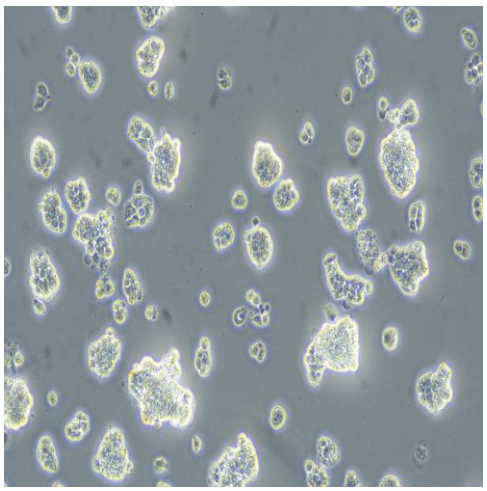


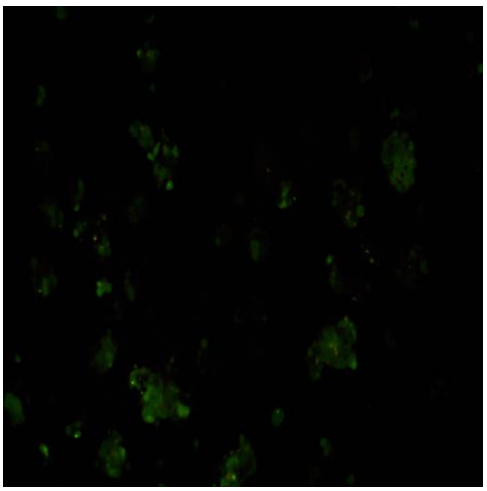
G.A (19.09 µg/ml)





S.A (1.51 µg/ml)





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C.A (12.71 µg/ml)