PONDICHERRY CENTRE FOR BIOLOGICAL SCIENCE AND EDUCATIONAL TRUST

Reg.No.2840/B4/2016 Pondicherry

GST IN: 33AADTP1541L1ZL

SUSTAINABLE RESEARCH FOR BETTERMENT OF MANKIND

CERTIFICATE OF ANALYSIS

PCBSET/2021-2022/RN00127	Date: 15/03/2022

User information: **Ms. P.S. Umabharathi,**
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India

Sample information: Powder

Sample Code: AM1 & Mercuric Chloride

Analysis: Fluorescence Bio imaging Assay_HeLa

Date of sample received: 04/02/2022

Date of Analysis: 01/03/2022

Date of report: 15/03/2022

Methodology:

Cell culture and Bio imaging:

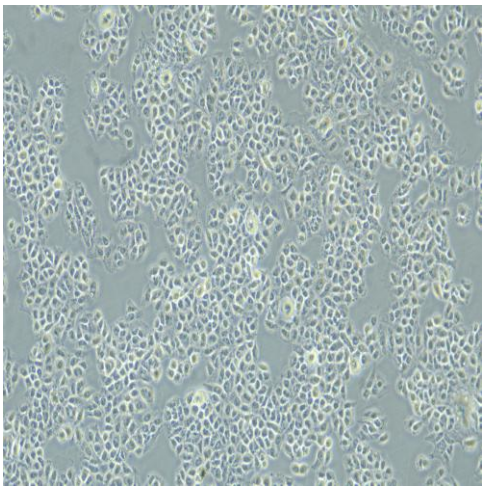
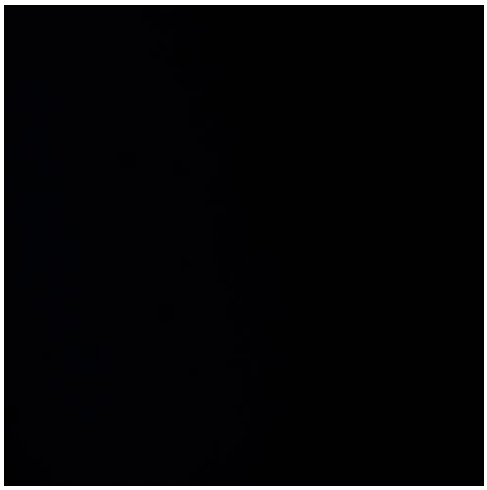
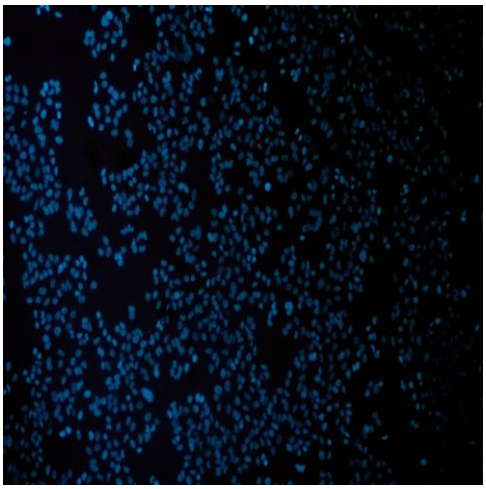
The Human Cervical Cancer cell line (**HeLa)** were seeded in a 12 well plate with the concentration of 1×10^5cells/well in MEM media with 1X Antibiotic Antimycotic Solution and 10% fetal bovine serum (Cat No: RM9955,Himedia, India) in CO_2 incubator at 37˚C with 5% CO_2 for 24 hr. Subsequently, the cells were washed twice with 1 ml of sterile 1X PBS, then the cells were treated with the samples AM_1 (10µg/ml) and Mercury Chloride (30µg/ml) in serum free media and incubated for 1 hr. Then, the cells were washed with sterile 1X PBS .Separate media control was maintained. Fluorescent imaging was done immediately using Optika IM-3FL4 fluorescent microscope (Optika, Germany) using UV filter.

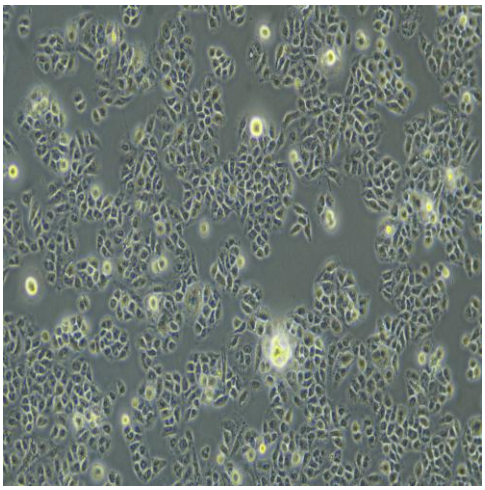
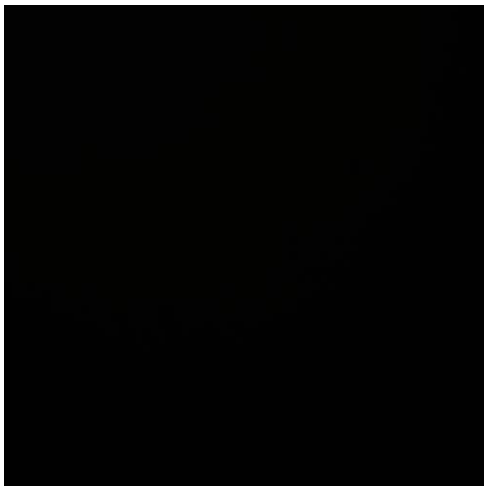
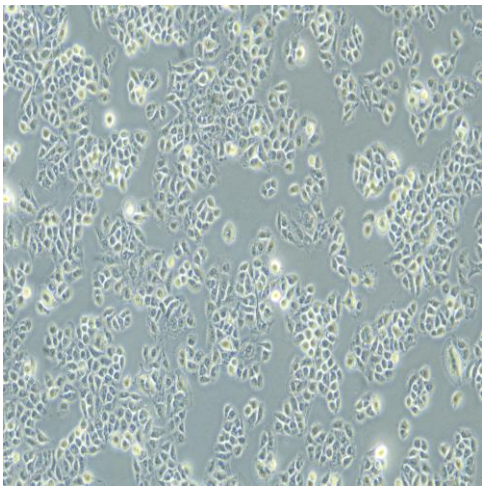
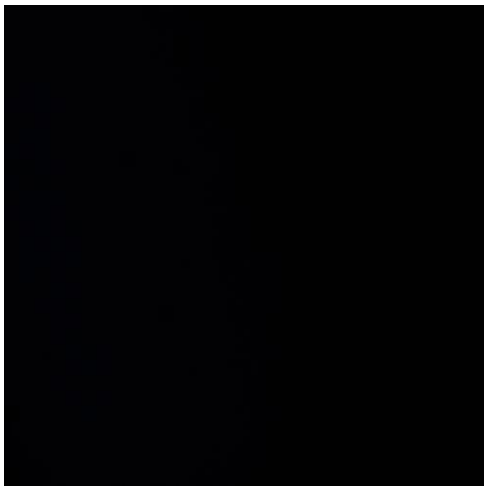
After completion of experiment, the control wells were used for DAPI staining and the same was considered as an internal control for fluorescent microscopic analysis as described by Elumalai et al. (2012). The media was removed; the wells were washed thrice with PBS and fixed with 3 % paraformaldehyde for 10 min at room temperature. The fixed cells were then permeabilized using 0.2 % Triton X-100 in PBS for 10 min, incubated with DAPI (0.5 µg ml^{-1}) for 5 min and viewed under Optika IM-3FL4 fluorescent microscope (Optika, Germany) using UV filter.

Reference

Elumalai P, Gunadharini DN, Senthilkumar K, Banudevi S, Arunkumar R, Benson CS, Sharmila G, Arunakaran J (2012) Induction of apoptosis in human breast cancer cells by nimbolide through extrinsic and intrinsic pathway. Toxicol Lett 215:131–142

Results

Control		
Control (Using DAPI stain)		

AM1		
Mercury Chloride		

AM1+ Mercury Chloride