Localization in Cells and NADPH Oxidase Activity in Glucose Induced Cytotoxicity on rat Muscle Cell Line

2. Materials and Methods

2.1 Chemicals and Reagents

DMEM/nutrient mixture F-12 Ham, fetal bovine serum (FBS), a, and fetal bovine serum (FBS) were purchased from Sigma Aldrich, India. Trypsin (4,5-dimethylthiazolyl-2)-3,5-diphenyltetrazolium bromide), and nicotinamide adenine dinucleotide phosphate (NADPH) were purchased from HiMedia L Limited, India. Dimethyl sulfoxide (DMSO), potassium dihydrogen phosphate (KH2PO4), potassium hydroxide (KOH), ethylenediamine tetraacetic acid (EDTA), and Triton X-100 were purchased from Central Drug House, India.

2.2 Plant Material

The Salacia oblonga powder (root and stem) was purchased from Nako Private Limited, Bangalore, India.

2.3 Cell Line and Culture Conditions

The L6 (rat skeletal muscle) cell line was purchased from National Science, Pune, India and was cultured by standard method. Wells were seeded at a density of 5x10^5 cells/well (Dyebulk 96-well cell culture plate and cultured overnight at 37°C under a humidified atmosphere prior to treatments). The cells were exposed to glucose at 75 mM for 24 hrs in order to determine its toxic dose for attaining death. The viability of the cells was determined by measuring the amount of formazan formed due to reduction of yellow NADH dehydrogenase inside the cells.

2.4 Effect of High Glucose on the Viability of L6 Cells

The L6 cells were seeded at a density of 5x10^5 cells/well (Dyebulk 96-well cell culture plate and cultured overnight at 37°C under a humidified atmosphere prior to treatments). The cells were exposed to glucose at 75 mM for 24 hrs in order to determine its toxic dose for attaining death. The viability of the cells was determined by measuring the amount of formazan formed due to reduction of yellow NADH dehydrogenase inside the cells.