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Oxidative Stress and Genotoxic Evaluation of Ethanolic Extract of *Azadirachta indica* on Thp-1 Cell Line

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ABSTRACT: Plant-based medicines are quite useful in the treatment of cancer. Neem is historically well known in Asia and Africa as a versatile medicinal plant with a wide spectrum of biological activities. The objective of the study was to screen the anticancer activity of Azadirachta indica in ethanolic extract on THP-1 cell line. The ethanolic extract of neem, prepared by dissolving 0.02 gm of neem in 40% ethanol, was analysed for its cytotoxic effect against THP-1 cell line. To estimate the cytotoxic effects of extract, cells were treated at four different concentrations (1000 µg/ml, 100 µg/ml, 10 µg/ml and 1 µg/ml). Further, MTT and NRU assay was used to estimate the extract induced cytotoxicity. Dyes (MTT and NRU) were added 2 hours prior to endpoint of the assay. After the endpoint of the assay media was removed and cell were solubilized in the lysing buffers and the plate was then read under the microplate reader at 550 and 660nm. The ethanolic extract showed significant cytotoxicity ($P \le 0.5$) at a concentration of 100 µg/ml. The cells were also analysed for apoptosis under the effect of Azadirachta indica extract with DNA fragmentation assay, and for this firstly DNA isolation was done and was quantified using Nanodrop Spectrophotometer. Then 100ng DNA samples were subjected to Agarose Gel Electrophoresis and the gel was visualized on the Gel Documentation system and analysed for fragmented DNA. The entire process demonstrated the cytotoxic effect of Azadirachta indica on THP-1 cell line and it can be concluded that if the dose range was further refined within the range of 100-1000 µg/ml there could be dose at which the entire population of the THP-1 cell line would be apoptosis induced. Further, apoptosis was studied with the ROS production and mitochondrial membrane potential measurements. In relation with the cytotoxic effects and DNA fragmentation assay, extract was able to induce ROS in the cells from 30 minutes of exposure and significant lowering of mitochondrial membrane potential. © 2016 iGlobal Research and Publishing Foundation. All rights reserved.

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