**Effects of 2,4-Dichlrophenoxy acetic acid (2,4-D) on Viability and Apoptosis of rat Peripheral Nerve derived Fibroblasts**


## Abstract

**Objective:** 2,4-dichlorophenoxyacetic acid (2,4-D) is a synthetic auxin which selectively kills broadleaf weeds in residential and agricultural environments. There is increasing concerns about 2,4-D exposure and the risk of developing pathological conditions on peripheral nervous system such as axonal degeneration and polyneuropathy. However, there is still a lack of appropriate in vitro and in vivo evidence addressing the mechanisms of 2,4-D damage on peripheral nerves. This project was accomplished to scrutinize the machinery of cytotoxicity of 2,4-D on peripheral nerve cellular elements.

**Methods:** Peripheral nerve fibroblasts, Schwann cells, HGF2, HUVEC and PC12 cells were treated with increasing concentrations of 2,4-D from 0.1μM to 5mM for 48 hours. Cell viability and proliferation were examined with MTT assay and cumulative cell counting, respectively. Flow-cytometric analyses were used for denoting the distribution of cells in different stages of cell cycle. Morphological events in damaged cells were determined using DAPI sand Acridine orange and Ethidium bromide staining. Apoptosis was investigated via measurement of Caspase-3/7 activity. SOD, GPx and MDA levels were measured to track biochemical changes after 2,4-D administration. Data were analyzed using one-way ANOVA and Student's t-test.

**Results:** The viability of all treated cells were significantly decreased following 2,4-D exposure in a dose dependent manner. 2,4-D caused cell cycle arrest in G1 phase. Morphological evaluations; using DAPI and AO/EB staining, indicated the increase in early and late apoptotic cell number with increase in concentration of 2,4-D. Apoptosis induction was quantified by higher activity of Caspase-3/7 enzymes; as executioner of programmed cell death. Furthermore, GPx activity and MDA level were augmented and SOD level was decreased following 2,4-D exposure.

**Conclusion:** Our results demonstrated that 2,4-D is toxic to peripheral nerve cellular elements in culture condition. 2,4-D toxicity is dose dependent where lower concentrations could inhibit

proliferation and higher concentrations induce apoptosis in peripheral nerve fibroblast and other cells investigated. Our data provides evidence; at least partially, that the loss of peripheral nerve homeostasis due to blood- nerve interface failure would be a primary mechanism of nerve damage following 2,4-D exposure.

**Keyword:** Peripheral nerve fibroblasts, Schwann cells, 2,4-D, Cell growth, Apoptosis