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Landfill Soil Leachates Induced DNA Damage and Apoptosis via Alterations in the Expression of Genes Associated with Apoptosis in Lymphoma Cell

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Landfill soil leachates, containing myriad of xenobiotics are known to increase DNA damage and cytotoxicity in organisms. However, possible molecular mechanism of leachates induced cytogenotoxicity is not clearly understood. The study here in investigated the possible mechanisms of DNA damage induced by Olusosun (OSL) and Nagpur (NPL) landfill soil leachates in lymphoma (Jurkat) cells. Jurkat was incubated for 24h with sub-lethal concentrations of the LC_{50} of OSL (22.04%) and NPL (24.89%). After treatment, cells were analyzed for DNA damage (alkaline comet assay), DNA fragmentation (agarose gel electrophoresis) and apoptosis (Hoechst 33258 – PI staining). Complementary DNA expression profiling of pro-apoptotic and anti-apoptotic genes regulating apoptosis in Jurkat cells were analyzed using real time PCR (RT-PCR) method. Agarose gel electrophoresis revealed that OSL and NPL induced significant increase in tail length, percentage tail moment and Olivetail moment and DNA fragmentations in the treated cells compared to the control. Hoecsht-33258 and PI based apoptotic analysis confirmed apoptosis in OSL and NPL exposed cells, via the presence of red and blue fluorescence in the treated cells. RT-PCR analysis revealed that OSL and NPL up-regulated pro-apoptotic genes and down-regulated anti-apoptotic gene with different fold changes. There was significant increase in fold change of AIFM2 (NPL=1.14 fold; OSL=1.46 fold), FADD (NPL=2.05 fold; OSL=1.47 fold), Caspase-2 (NPL=1.67 fold; OSL=4.79 fold), Caspase-6 (NPL=1.05 fold; OSL=1.75 fold), BID (NPL=1.17 fold; OSL=1.05 fold), p53 (NPL=1.22 fold; OSL=3.10 fold), BAD (NPL=2.13 fold; OSL=5.65 fold) and down-regulation of AP15 (NPL= -1.58 fold; OSL= -1.22 fold) in treated cells compared to the control. Apoptotic analysis confirmed that the cells were in late apoptotic phase, suggesting that the cell membrane was no longer intact hence PI stained the cell's chromatin materials. The altered genes suggest instability in the intrinsic/extrinsic pathways involved in the regulation of apoptosis. Toxic metals Cd, Pb, Cr, Co and 32 different PAHs and PCBs were detected in the OSL and NPL. These possibly induced the observed DNA damage and apoptosis in Jurkat cells via alterations in apoptotic pathways.

Keywords: Apoptotic genes, Comet assay, DNA fragmentation, Landfill soil leachates, Lymphoma cell.