



SYNERGISTIC EFFECT OF FLUORIDE AND HARDNESS OF DRINKING WATER ON NEPHROTOXICITY; CYTOTOXICITY ASSAY USING VERO CELLS

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Abstract

Chronic Kidney Disease of Unknown Aetiology (CKDu) is viewed as a non-communicable disease and it is responsible for high mortality in rural farming communities in North Central province. Increasing shreds of evidences on this phenomenon suggest that high concentrations of fluoride and hardness in drinking water induce renal injury. The present study was focused to determine the synergistic effect of fluoride and hardness by evaluating the cytotoxicity and possible apoptotic effect on epithelial kidney cells using Vero cell line (ATCC® CCL-81™). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was done to determine the cell viability and cells were seeded in 96-well plates as 5×10^3 cells per well. Cells in different wells were exposed to different concentrations of fluoride (0.5, 2.5, 5.0, 7.5, 10.0, 12.5, 15.0 mgL⁻¹) hardness (60, 100, 200, 400, 600, 800, 1000 mgL⁻¹) and fluoride: hardness ratios (0.5:60, 2.5:100, 5.0:200, 7.5:400, 10.0:600, 12.5:800, 15.0:1000 mgL⁻¹). The assay was performed in triplicates and IC₅₀ values of fluoride, hardness alone and fluoride:hardness ratios on the Vero cells were determined. Mortality rates of the cells exposed to fluoride concentration series were ranged from 10.90% to 37.12% while the cells exposed to hardness concentrations were ranged from 13.69% to 37.70% indicating increment with the high concentrations. Further, the mortality rate was increased from 11.11% to 49.09% in the cells exposed to fluoride and hardness ratios. IC₅₀ values of fluoride, hardness alone and fluoride:hardness ratios were recorded as 20.34, 1479.53 and 16.19:1045.29 mgL⁻¹ respectively. When IC₅₀ values were compared, the significantly higher value was observed in the cells exposed to fluoride alone than the cells exposed to fluoride:hardness ratio ($p < 0.05$), as well as significantly higher value, was observed in the cells exposed to hardness alone than the cells exposed to fluoride:hardness ratio ($p < 0.05$). Thus, the significant increment in cell mortality rate in cells exposed to fluoride:hardness ratio indicates the nephrotoxic effect. Therefore, the synergistic effect of fluoride and hardness may contribute to the CKDu.

Keywords: fluoride, hardness, MTT assay, cell mortality, Vero cells