Abstract

The use and abuse of psychoactive drugs continue to escalate at an alarming rate worldwide, with South Africa identified as one of the leading drug regions in the world. The introduction of a new designer drug, ‘nyaope,’ has worsened the overwhelming increment in the drug use problem in South Africa. Nyaope, is a highly addictive illegal psychoactive cocktail drug presented as a fine white to brown powder, containing large amounts of heroin and other substances such as rat poison, caffeine, anti-retroviral drugs (ARVs) and detergent powder. Their contribution to the toxic effects of nyaope remains unknown despite literature reporting the detrimental effects of some of them.

Our study design included both *in vitro* and *in vivo* experiments. For *in vitro* experiments HepG2 cells were used as surrogate liver cells and SH-SY5Y cells represented neurons. These cells were treated with increasing concentrations of nyaope and the effects thereof on cell morphology, cell viability and cell death were subsequently investigated at various time points. For the *in vivo* experiments, Wistar rats received intraperitoneal injections of nyaope for 3 consecutive days. On day 3 liver tissue and the prefrontal cortex were dissected and subsequently analyzed by polymerase chain reaction (PCR) methodology for the presence of cell death markers.

Our data showed that nyaope caused a significant decrease in cell confluence and aberrant adherence with many cells floating in the cultured medium, suggesting that nyaope created an unfavourable growth environment for the cells. This observation was supported by a nyaope-induced, concentration dependent, decrease in cell viability in both HepG2 and SH-SY5Y cells. Measurement of lactase dehydrogenase (LDH) activity showed that nyaope exposure resulted in necrotic cell death in SH-SY5Y cells. The evidence for an apoptotic form of cell death caused by nyaope was less convincing with mixed findings observed for the markers (Bax/Bcl-2) studied. Our data further suggested that the autophagy process could have been partially initiated after nyaope exposure as increased p62 and reduced LC3, at the 24-hour time point, were recorded.

Results from the *in vivo* experiments reflect the *in vitro* findings with some evidence of the activation of apoptotic and autophagic pathways being observed, albeit differently in the two tissue types studied.

To our knowledge, this is the first study to determine the effects of nyaope on cell viability and death, with a focus on the liver and the brain. Nevertheless, our findings indicate that nyaope may have deleterious effects on these organs that may contribute to its malfunctioning. However, more studies are required to expand on these findings to create a more complete picture of nyaope toxicity.