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Investigating Acute Cytotoxicity of Misused Prescription Drugs in Sri Lanka Using the *Allium cepa* Bioassay: A Study on Tramadol & Pregabalin

Athapaththu AAMK¹, Indudunu GMK¹, Madusanka HGRL¹, Dissanayaka CUS¹, Thiwanka JLPT¹, De Silva ULRM¹, Madhuranga HDT^{1,2}, Jayamaha AR²

¹Department of Biomedical Science, Faculty of Health Sciences, KIU, Sri Lanka

²Research and Innovation Division, KIU, Sri Lanka

Background: Tramadol and Pregabalin, prescription medications, are widely misused by Sri Lankan drug addicts. This assay was conducted as a preliminary study. The findings from such studies enable the evaluation of the acute toxicity of Pregabalin and Tramadol using animal models.

Objective: This study aimed to determine the acute cytotoxicity of Pregabalin and Tramadol using the *Allium cepa* bioassay.

Method: Tramadol and Pregabalin, commonly misused prescription drugs among drug addicts in Sri Lanka identified through a literature review. Stock solutions (50 mg/50 mL distilled water) were diluted into a two-fold series (2×10^{-3} – 1 mg/mL) using pharmacy-grade drugs. Uniform *Allium cepa* bulbs with ~1 cm roots, pre-germinated in dechlorinated water for 48 hours at $27 \pm 2^\circ\text{C}$, were exposed to these concentrations for 96 hours. A 5% DMSO solution served as the positive control, and dechlorinated water as the negative control. Root lengths and morphological abnormalities (e.g., gelling, necrosis, hooks, swelling) were recorded post-exposure. The experiment was triplicated, and data were analyzed using one-way ANOVA with statistical significance set at $p < 0.05$.

Results: Tramadol and Pregabalin exhibited dose-dependent inhibition of *Allium cepa* root growth, with EC_{50} values of 0.48 mg/mL ($R^2 = 0.9934$, $p < 0.0001$) and 1.32 mg/mL ($R^2 = 0.9930$, $p < 0.0001$), respectively. Maximum root lengths were observed at 32×10^{-3} mg/mL for Tramadol (6.13 ± 0.04 cm) and 2×10^{-3} mg/mL for Pregabalin (7.4 ± 0.04 cm), compared to the control 7.2 cm. Morphological abnormalities, notably hook formation, were consistently observed in *Allium cepa* roots treated with drug concentrations (2×10^{-3} mg/mL - 1 mg/mL). Specifically, at certain concentrations within this range, 50% of the roots exhibited hook formation, indicating potential drug-induced cellular stress or toxicity.

Conclusion: Tramadol and Pregabalin cause dose-dependent cytotoxicity and morphological changes in *Allium cepa*, indicating minimal cytotoxic effects. Further in vivo studies are needed to assess their acute toxicity using a compatible animal model.

Keywords: Acute toxicity, *Allium cepa* assay, Pregabalin, Prescription medication, Root growth inhibition, Tramadol