

A Comparative Study on the Toxicity and Biocompatibility of *Hemidesmus indicus* Aqueous Extract-in Various Developmental Stages of Zebrafish *Danio rerio* using the FET293 Bioassay

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Abstract

Hemidesmus indicus R. Br. (Periplocaceae) commonly known as “Iramusu” in Sinhala; found in Sri Lanka is used for a variety of ayurvedic preparations. However, the toxicity of *H. indicus* aqueous plant extract remains unknown. The purpose of this study was to investigate the biocompatibility and toxicological properties of *H. indicus* aqueous plant extract using zebrafish embryo assay (FET293 bioassay) following OECD guidelines. Wild-type male and female zebrafish were maintained at 27.0°C, pH (7 ± 0.5) and nitrates (<0.009 g L⁻¹), nitrite (8-12 g L⁻¹), and ammonia levels (<0.05 mg L⁻¹) were monitored weekly. Aerial parts of the plant were extracted into distilled water using the maceration method. The LC50 in the brine shrimp lethality assay was 48.2 mg/mL, which was used to determine the concentration range for the zebrafish embryo assay. Two- fold dilutions ranging from 8-125 mg/mL were prepared based on brine shrimp lethality assay results. The study was conducted according to the KIU Ethics Review Committee approval. Zebrafish embryos were exposed to the plant extract, and hatch rate, mortality rate, survival rate, heart rate, and developmental deformities were observed at specific time points. Statistical analysis was performed using GraphPad Prism 9 software. Hatchability increased with extract concentration, reaching the highest at 8 mg/mL at 96 hpf. Survival rate decreased with higher concentrations, reaching 0% at 63 and 125 mg/mL from 72 hpf onwards. Heart rate remained within the normal range except at 63 and 125 mg/mL, in which 100% mortality was observed. Developmental deformities were observed, including yolk sac edema and pericardial edema, mainly at higher concentrations. The *H. indicus* extract was safe at 8 mg/mL but exhibited mild- moderate toxicity effects at higher concentrations.

Keywords: *Biocompatibility, Hemidesmus indicus, Toxicity, Zebrafish embryos*