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**Developmental and teratogenic toxicity of Alstonia *scholaris (L.) R. Br.* ethanolic crude extract in zebrafish (*Danio rario*) embryo**

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**Abstract**

*Alstonia scholaris (L.) R. Br.* of *Apocynaceae* family, commonly known as “Devil’s Tree”, has ethnomedicinal importance in the treatment of various diseases like asthma, cancer, jaundice, leprosy, and malaria. *A. scholaris* showed potentiality as an antibacterial, antiviral, antioxidant, anti-fungal agent in various studies. In the present study, the antimicrobial activities of *A. scholaris* ethanolic extract were determined using the broth microdilution method. The developmental and teratogenic toxicity effect of EE of *A. scholaris* was also determined in zebrafish embryos at 500, 250, 125, 62.5, 31.25, and 0 µg/ml concentrations. The toxicity study was done following the OECD guideline 236 for zebrafish acute toxicity testing. Zebrafish embryos were treated with *A. scholaris* EE at different concentrations at 2 hours post fertilization (hpf). During 5-day exposure (till 96 hpf) with EE, developmental and teratogenic features like egg coagulation, mortality, somatic development, eye formation, hatching, yolk sac and pericardial edema, pigment formation, movement, and heartbeats were recorded every 24 hrs. The results demonstrated that ethanolic extract (EE) of *A. scholaris* showed antimicrobial activity against the fish pathogen *Pseudomonas fluorescens* with a half-minimum inhibition concentration (MIC50) of 46 µg/mlandshowed broad-spectrum activities against *Streptococcus iniae* (MIC50: 67 µg/ml) and *Flavobacterium columnare* (MIC50:12 µg/ml). During EE exposure for toxicity test with zebrafish embryos, at 24, 72, and 96 hours post-treatment (hpt), mortality was observed 10%, 20%, and 40%, respectively at 500µg/ml concentration. At 96 hpt 15% mortality was observed in 250 µg/ml concentration; no mortality observed in other concentrations. At 96 hpt, hatching efficiency was reduced by 25% in 500µg/ml concentration. Malformation of varying levels was visible at higher concentrations (125-500 µg/ml). In 500 µg/ml, 100% of embryos were found malformed with various features such as yolk sac edema, pericardial edema, body bending, tail bending, and short body length. No observed-adverse-effect (NOAEL) was observed at 62.5 µg/ml. LC50 of *A. scholaris* EE found 254 µg/ml and EC50 276 µg/ml. The teratogenic index (TI) value, the ratio of LC50 and EC50, was found less than 1 indicating *A. scholaris* EE is notteratogenic for zebrafish embryos and can be used for further aquaculture welfare and drug development studies.

Keywords: Aquaculture, antibacterial activity, zebrafish embryo toxicity, *Alstonia scholaris.*