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Characterization of tissue specific cholinesterases in native fishes, *Rasbora daniconius* and *Dawkinsia singhala*: their sensitivity to the heavy metal, copper

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Aquatic pollutants could pose health hazards to humans and wildlife. Cholinesterases (ChEs) have been recognized as biomarkers of exposure to a range of aquatic pollutants including organophosphate and carbamate pesticides and heavy metals. This study examined tissue specific characteristics of ChE activities in the brain, muscle and gills of two native freshwater fish species *Rasbora daniconius* (Sinhala; Dandiya) and *Dawkinsia singhala* (Sinhala; Dankola pethiya) in Sri Lanka with the aim of using ChEs of these fishes as potential biomarkers for biomonitoring aquatic pollution. The main objectives were to characterize the biochemical properties of ChEs in brain, muscle and gill tissues of *R. daniconius* and *D. singhala*, using two specific substrates and three selective inhibitors and to measure *in vitro* sensitivity of the ChE activities to exposure to heavy metal, Copper. Cholinesterase activity measurements with two substrates (acetyl thiocholine iodide (ATCI) and butyryl thiocholine iodide (BTCI)) showed the presence of two different cholinesterases in both fishes namely Acetyl cholinesterase and Butyryl cholinesterase. With the estimated kinetic parameters of the enzymes (K_m, V_{max} and V_{max}/K_m) for both substrates, highest catalytic efficiency was obtained for brain tissues with the two substrates for both fish species. Of the two substrates tested, the catalytic efficiencies were generally higher when ATCI was used as the substrate in crude enzyme extracts (V_{max}/K_m for brain ChE: 3.95 for *R. daniconius;* 2.04 for *D. singhala*). Using enzyme inhibition pattern in response to three selective inhibitors (Eserine, BW254C51 and Iso-OMPA), it was found that the brain and muscle tissues of both fishes contained mainly Acetylcholinesterase enzyme whereas the gill tissues are the sources of both Acetylcholinesterase and Butyryl cholinesterase enzymes. The results of the present study clearly proved that ChE activities of both species have a concentration depended inhibition pattern in response to *in vitro* exposure of Cu^{2+} at least in the tested concentration range (0.05-2 mM). ChEs in the gill tissues in both fish species are more sensitive to the Cu^{2+} than the ChEs in brain and muscle tissues. Overall results indicate that cholinesterase enzymes of *R. daniconius* and *D. singhala* may be used as biomarkers for neurotoxic contaminants such as the heavy metal, copper. Crude extracts of the ChE enzymes in brain, muscle and gill tissues of the two fish species can be used in routine procedures for screening anticholinesterase contaminations in water resources.

Keywords: Acetylcholinesterase, biomarkers, butyryl cholinesterase, *Dawkinsia singhala, Rasbora daniconius*