

Application of Acetylcholinesterase Activity in Marine Organisms as a Biomarker of Coastal Pollution

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Abstract:

The marine environment is continually being polluted by different types of neurotoxic contaminants like organophosphorus, carbamate insecticides, heavy metals and various other neurotoxins from various sources along the coasts. These neurotoxic compounds adversely affect the health of the marine ecosystem by inhibiting the acetylcholinesterase (AChE) activities of various species of marine organisms. This paper presents the effective use of acetylcholinesterase activities in marine gastropod, *Cerethedia cingulata* from different sites along the Goa coast as a useful biomarker technique for evaluation of the ecotoxicological impact of neurotoxic compounds. The AChE activity was measured by the standard method using acetylcholine bromide as the substrate and bromothymol blue as the indicator in phosphate buffer medium. The enzyme activity was measured

in terms of nanomoles of acetic acid liberated per mg of protein per minute from the enzyme-substrate reaction. The AChE activities of the marine gastropod showed wide variation along the Goa coasts viz: Palolem, 80.85, Tirakol, 78.38, Hollant: 54.784 and Dona Paula: 35.515. The results were compared with those from the reference sites at Palolem and Tirakol (AChE activity: 80.853 and 78.383 respectively). It provides an insight into the levels of contamination of the Goan coastal environment by neurotoxic contaminants.

Introduction:

Various types of contaminants are continually polluting the marine environment. Some of these contaminants belong to the class of neurotoxicants such as cadmium, lead, mercury, copper, chromium, organophosphorus pesticides, carbamate pesticides, organochlorine pesticides etc.

The marine organisms are being exposed to enhanced concentration of such anthropogenic pollutants leading to severe damage in their physiological system. Thus the level of contaminants of marine environment can be evaluated in terms of ecotoxicological impact molecular biomarker. (Sarkar A et al 2006; 2004; 1996; 1994.) The biomarker or in other words the biological responses of the organisms exposed to any kind of toxic compounds are manifested in terms of physiological changes such as inhibition / induction of enzyme activity, DNA strand breaks, loss of DNA integrity etc. In this regard, various types of biomarkers have been in used for biomonitoring of pollution for example acetylcholinesterase activity (AChE) is used for biomonitoring of neurotoxic compounds. Acetylcholine is an ammonium compound. It was the first transmitter substance to be isolated in 1920. (S.B. Mushigeri et al 2005) In 1964, the enzyme commission recommended [Acetylcholine Acetylhydrolase; E.C.3.1.1.7.], Acetylcholinesterase (AChE) as a 'true' and 'specific' cholinesterase, while the other cholinesterases were collectively termed as 'pseudo' [Acylcholine Acylhydrolase; E.C.3.1.1.8.]. The pseudocholinesterases included Butyrylcholinesterase (BChE) and Propionylcholinesterase (Ashwani V 1992). The enzyme acetyl cholinesterase (AChE) is considered as a suitable biomarker for detecting environmental pollution caused by neurotoxic compounds, such as organophosphates, carbamates and heavy metals (Galgani and Bocquene', 2000). The serine hydrolase AChE is responsible for the signal termination at cholinergic synapses by rapid hydrolysis of the neurotransmitter acetylcholine. The presence of neurotoxic

compounds results in a measurable inhibition of this enzyme .Organophosphate and carbamate insecticides are potent neurotoxins and exert their toxicity by blocking the breakdown of acetylcholine (ACh) by the enzyme, acetylcholinesterase (AChE).Inhibition of the AChE enzyme will result in a buildup of ACh causing a continuous and excessive stimulation of the nerve/muscle fibers which will result in tetany, paralysis and eventual death (Simone Pfeifera et al 2005).

Marine bivalves such as clams, Mytilid mussel and oysters, which represent the entire Indian coast and are sentinel species, are considered to be the best organisms for such studies .In view of the state of pollution in marine environment it is indeed of urgent need of the hour to evaluate the ecotoxicological impact of various types of contaminants in the marine environment using molecular biomarkers. (Brooks, K.N et al 2003 and Brown, C 2006)

Material and methods:

The work was carried out by selecting different sites along the Goa coast (Fig.1) according to the vulnerability of the regions to various types of pollutants, points of industrial discharge, waste disposal etc. A large number of biological (snails, fishes, clams etc.), water and sediment samples were collected from selected sites along the Goa coast for evaluation of the impact of neurotoxicants on the enzyme activity. Physico-chemical water parameters such as pH, salinity, temperature, conductivity, turbidity, nitrate, nitrite, dissolved oxygen, BOD etc. were standardized in order to determine the water quality of the sampling sites along the Goa coast.

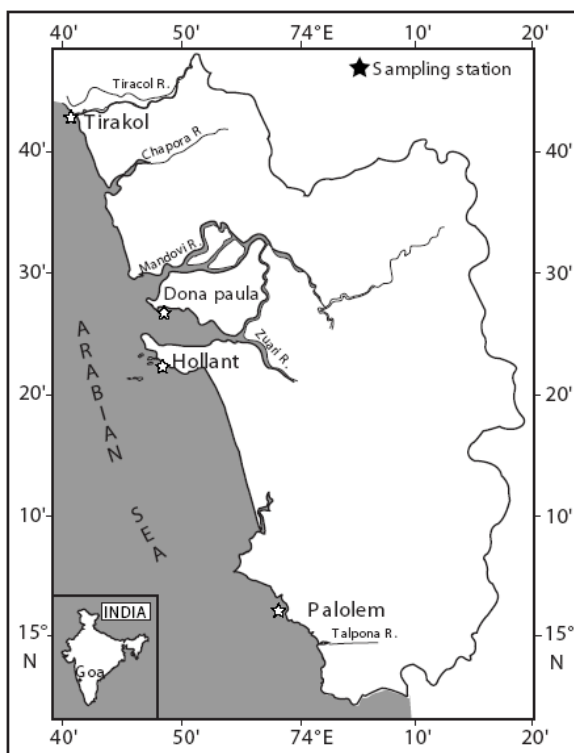


Fig.1. Locations of sampling along the Goa coast

Isolation of Acetylcholinesterase enzyme (AChE):

In order to isolate AChE enzymes the whole tissues were removed from the snails and pooled together to make composite samples. The composite samples were divided into duplicates for AChE study. From these samples 1 gm of tissue was taken in each homogenizing tube and the enzyme was isolated by homogenization with 0.01 M phosphate buffer (pH 7.4 – 0.1) spiked with Triton-X-100 (0.2%) and 0.25 M sucrose (1:1) solution using an Ultra Turrax homogeniser (T-25). The homogenates were centrifuged by refrigerated centrifuge (Etek) at 8000 rpm for about 35 min at 4°C. The protein concentration of each of the sample extract was determined according to Lowry's method (1951) using bovine serum albumin as the standard (A. Sarkar et al 2006).

Determination of AChE activity:

The AChE activity was measured following method using bromothymol blue as indicator and Acetylcholine bromide as the substrate for the reaction kinetics. (Sarkar 1992). The changes in pH (Δ -pH) was measured at an interval of 10 min over a period of 1 h of incubation corresponding to the amount of acetic acid liberated due to interaction with the AChE enzyme. The unit of activity of AChE was calibrated with respect to Δ -pH (changes in pH) against the nanomoles of acetic acid liberated due to hydrolysis of acetylcholine. The AChE activity is expressed in terms of units corresponding to nanomoles of acetic acid liberated per minute per mg of protein. (A. Sarkar et al 2006)

Results and discussion:

Four sampling sites Palolem, Hallent, Dona Paula and Tirakol, were chosen along the Goa coast depending upon their vulnerability to toxic contaminants. Palolem and Tirakol were taken as reference site as they are situated to the extremes of Goa Coast. Hallent and Donapaula were taken as experimental sites. The results obtained from these two sites where compared with the reference sites. Promising results were obtained with an AChE activity of 54.784 (nmol/min/mg) and 35.515 (nmol/min/mg) for Hallent and Donapaula respectively, as compared with the reference results of 80.853 and 78.383 (nmol/min/mg) for Palolem and Tirakol respectively. These results give a complete indication that the two sites Hallent and Donapaula are more polluted as compared to the reference sites Pallolem and Tirakol. Similar works have been carried out by various researchers throughout the world. AChE activity as a biomarker for neurotoxic

contaminants in various gastropods was studied by A Sarkar et al (2006). Another work on Acetylcholinesterase as a biomarker of exposure to neurotoxic compounds in the clam *Tapes philippinarum* from the Lagoon of Venice, was carried out by Valerio Matozzo et al (2005). All these results show a decrease in

enzyme activity with increase in neurotoxic contaminants. Hence, it is proved that AChE activity can be successfully used as a biomarker for the assessment of marine pollution.

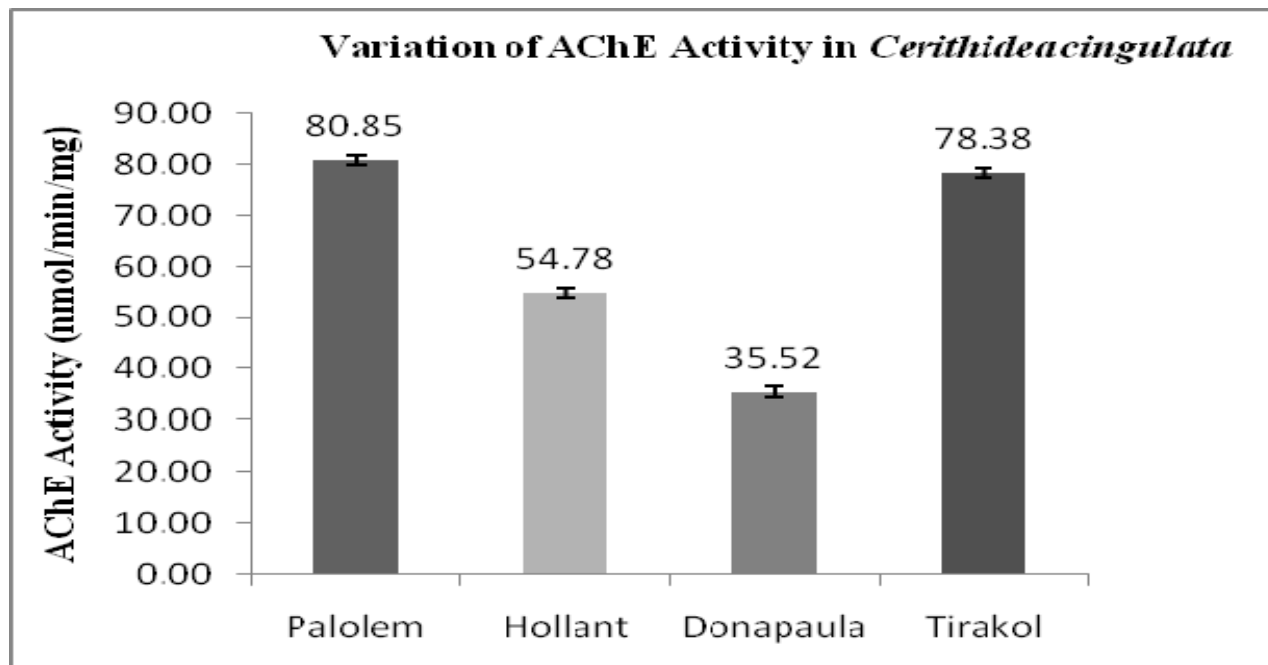


Fig. 2. Variation in AChE activities in marine gastropod, *Cerethedia cingulata* from different sampling sites

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