RESEARCH PAPER

Asian Journal of Bio Science (April, 2010) Vol. 5 Issue 1 : 77-79

Sub-lethal effect of pesticide, kingdoxa on ach and ach E activity of a fresh water mussel, *Lamellidens marginalis* (Lamark)

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(Accepted : February, 2010)

Acetyl cholinesterase (AChE) is an enzyme present in the synapses and myoneural junctions. The levels of the enzyme causes neuronal diseases. Many pollutants causes neuronal diseases to aquatic organisms. The objective of the study was to study the effect of pesticide 'Kingdoxa' in freshwater mussels *Lamellidens Marginalis* (Lamark). Therefore, the effect of sub lethal concentration of commonly used pesticide Kindoxa was studied on the Acetylcholine (ACh) and Acetylcholineserase (AChE) activity in foot, mantle and gill of a fresh water mussel, *L. marginalis.* To compare the effect of these pesticides on Ach and AChE activity, the mussels were exposed to sub-lethal concentration (Kindoxa 0.33 ppm) of pesticide for 48 hours. After the exposure, the animals were sacrificed tissues were isolated and processed for the estimation of Ach and AChE activity. When exposed to sublethal concentration of the pesticide, the activity levels of AChE was decreased and ACh content was increased in all the three tissues treated with Kindoxa. At tissue level inhibition was in the order of mantle > gill> foot. The inhibition of AChE and elevation of Ach content was higher in mussels exposed to Kindoxa. Therefore, it is possible that Kindoxa due to their inhibitory effect on the enzyme and ability to obstruct ACh hydrolysis cause its accumulation and ultimately lead to the disruption of nervous co-ordination and consequently death, in case of acute poisoning.

Key words : Kindoxa, Acetylcholine, Acetyl cholinesterase, *L. marginalis*

INTRODUCTION

Pesticides as pollutants is the distinction between the target species that pesticides and designed to kill and non-target species inhabiting the treated areas, that are not intended to be killed or affected in some un desirable way. Frequently it is not possible to kill just the target species; other co- existing species are also affected. Also, the more stable pesticides tend to move into parts of the environment where they were not intended to be, e.g. to ground water, or in the tissues of fishes and mussels like aquatic organisms, as in the case for the residues of most chlorinated pesticides. Organophosphate and carbamate insecticides degrade more rapidly after application than the organochlorines, they generally are more toxic before they breakdown. These compounds are effective insecticides but can have harmful effects on non-target organisms like aquatic organisms by inhibiting acetylcholinesterase (AChE) activity (Grue *et al.*, 1991). Therefore, it is the unintentional aspects of pesticide use that result in environmental pollution.

Acetyl cholinesterase (AChE) is an enzyme present in the synapses and myoneural junctions. It is a membrane bound enzyme situated near the vicinity of acetyl choline receptors, which act as ligand gated channels, and hydrolyses acetyl choline into acetate and choline. Majority of the insecticides inhibit the activity of AChE, there by prolong the action of acetyl choline leading to death (Aldridge and Devison, 1953). Hence, the study of the inhibition of AChE indicates the level of toxicity and extent of pollution of the pesticides (Metcalf, 1971; O'Brien, 1977).

Reports are available on the inhibition of AChE by a verity of pesticides on aquatic invertebrates and fishes. (Jagan, 1991; Singh and Kumar, 2000; Tilak *et al.*, 2003; Tilak *et al.*, 2005; Vijayendra Babu *et al.*, 2005; Shakthivel, 2006; Ganesh *et al.*, 2006). There is a paucity of information on the effect of pesticides on AChE activity of freshwater mussel, *Lamellidens marginalis* which is found in all fresh water ecosystems of the region. These animals are bottom dwelling and circulate large amounts of water through their bodies by filter feeding mechanism to obtain food and oxygen and there by accumulate the pesticides in their bodies. Hence, the study of ACh and AChE activity of mussel indicates the extent of pollution. The present study deals with the effect of pesticide Kindoxa on the AChE and ACh activity in certain tissues of *L. marginalis*.

MATERIALS AND METHODS

Fresh water mussels, *L.marginalis* were collected from local ponds and brought to the laboratory in plastic buckets containing same pond water. They were acclimatized to the laboratory conditions before experimentation. Commercial grade pesticide Kindoxa

was used as they are used locally in the cotton fields LC50 values were obtained by exposing the animals to different concentrations of pesticides for 48 hours under static bioassay conditions and the value was caluculated according to Finney (1952) modified by Busvine (1971). To compare the effect of these pesticides on Ach and AChE activity, the mussels were exposed to sub-lethal concentration (Kindoxa 0.33 ppm) of pesticide for 48 hours. After the exposure, the animals were sacrificed tissues were isolated and processed for the estimation of Ach and AChE activity. The acetylcholine (Ach) content was estimated by the method of Hestrin (1949) as modified by Augustinson (1957).The acetylecholinesterase (AChE) levels were estimated by the method of Metcalf (1951).

RESULTS AND DISCUSSION

The results of Ach and AChE activity from control and pesticide treated tissues of *L. marginalis* are presented in Table 1. The ACh content showed an increase in all the three tissues treated with Kindoxa. At tissue level inhibition was in the order of mantle > gill> foot.

Table 1 : Changes in AChE activity and ACh level in the selected tissues of control and pesticide exposed mussel, *Lamellidens marginalis* (Lamarck). Each value is mean ± S.D. of 8 individual observations

	AChE*		Ach**	
	Control	Kingdoxa	Control	Kingdoxa
Foot	1.75 ± 0.152	1.58 ± 0.145 PC = - 9.71	2.96 ± 0.182	3.89 ± 0.098 PC = + 31.41
Mantle	1.19 ± 0.148	0.92 ± 0.081 PC = - 22.68	2.29 ± 0.221	3.09 ± 0.113 PC = + 34.93
Gill	1.46 ± 0.132	1.23 ± 0.121 PC = - 15.75	3.17 ± 0.126	4.39 ± 0.179 PC = + 38.48

P<0.05 ('t' test), PC = Per cent change, (-) sign indicates decrease over control.
* Values are expressed as µmoles of ACh hydrolysed/100 mg wet weight of the tissue/h.
** Values are expressed as µmoles/100 mg wet weight of the tissue.

Several studies have already documented the inhibition of AChE enzyme activity due to lindane intoxication (Rodrigyez *et al.,* 1987; Bano and Bhatt., 2007). Inhibition of AChE and accumulation of ACh by phosphomidon, Dimethoate and Ziram has been reported in fresh water mussel, *Lamellidens marginalis* (Lamarck) (Vijayendra Babu, *et al.,* 2005). Decrease in AChE activity is in agreement with earlier reports in tissues of *Lamellidens marginalis* exposed to individual and joint effects of endosulfan and fenvalerate, it is possible to conclude that only one of the toxicants in the mixture is able to cause mortality and which is likely to be Endosulfan (Jagan, 1991).

The exposure of sublethal concentration of organophophates on the activity levels of AChE were found to decrease in all the tissues of the fish, *Cattla cattla, Labeo rohita* and *Cirrhinus mrigala* (Tilak *et al.,* 2005). With organophosphate, phosphorylation of AChE (formation of phosphorylated enzyme) is progressive with time and its hydrolysis to yield normal enzyme is slow. This explains the increased inhibition of AChE in liver of Malathion exposed fish, *Catla catla* (Singh and Kumar, 2000). In the brain tissue of Indian bullfrog, *Haplobatrachus tigerinus* (Daudin) the maximum inhibition of AChE was noticed from 6 hours to 12 hours on exposure to Fenvalerate (Tilak *et al.,* 2003).

Based on the experiments on insects and mammals there has been a wide spread acceptance of the view that AChE is indeed the true target of organophosphates and carbomate poisoning process (O'Brien, 1961). Consistent with the inhibition of AChE, accumulation of ACh was evident in all the tissues observed. Disruption of nervous activity by the accumulation of ACh at the neuromuscular synapses was opined by several workers (O'Brien, 1977; Fukuto, 1971; Metcalf, 1971). Therefore, it is possible that Kindoxa due to their inhibitory effect on the enzyme and ability to obstruct ACh hydrolysis cause its accumulation and ultimately lead to the disruption of nervous co-ordination and consequently death, in case of acute poisoning.

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