Research Article

STUDIES ON INFLUENCES OF *DIMETHOATE* (AN ORGANOPHOSPHATE PESTICIDE) ON *BRAIN* ACETYLCHOLINESTREASE ACTIVITY IN COMMON CARP, *CYPRINUS CARPIO COMMUNIS*

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ABSTRACT

Influences of Dimethoate on brain acetylcholinestrease (AChE) activity of common carp, *Cyprinus carpio communis* was observed under *in vitro* conditions. The exposure concentrations started from 0.80 mg/l, 1.15 mg/l and 1.45 mg/l as 50%, 70% and 90% of the LC_{50} value of 1.70mg/l respectively for 96hr. Statistically significant and dose-dependent reductions in the mean AChE activities was seen. The results show that test animal brain acetylcholinestrease activities were significantly inhibited by sub lethal doses of Dimethoate. Inhibition of brain AChE will definitely cause physiological and behavioral modifications that reduce survival ability of the animals at any stage of growth.

Keywords: Dimethoate, acetylcholinestrease, *Cyprinus carpio communis.*

INTRODUCTION

Pesticides are employed routinely in the integrated farming practices to protect crops and animals from insects, weeds and diseases. Liberal use of pesticides at different stages of crop production starting from seed processing to storage of agricultural produce is posing great danger to aquatic environment. These pesticides are carried into aquatic ecosystem by surface run-off from sites of application, where they enter into the organisms through food webs and also through contact in water. Therefore, the health of aquatic ecosystem is being adversely affected because they serve as ultimate link for these pesticides.

Because of the environmental longevity and toxic effects of organo-chlorines, the agriculture industry has increasingly relied upon organo-phosphate pesticides (Rangarsdottir, 2000)[1]. These pesticides are presumed to be safe due to their rapid environmental degradation (relative to organo-chlorines). Although organo-phosphates are generally less persistent and bioaccumulative than organo-chlorines, which may have relatively high toxicities and are acutely toxic to a wide variety of non target organisms (Cowman and Mazantis, 2000)[2].

Organophosphates are neuro toxins that disrupt the central nervous system of animals by inhibiting the enzyme acetylcholinestrease (Rangarsdottir, 2000)[1]. The responsiveness of animals including fishes to organophosphate compounds ranges from altered metabolic activities to depending upon the level of exposure death (Anam and Mitra, 1995)[3].

Among different classes of organo-phosphate pesticides Dimethoate is commonly used pesticide. It was first described by Hoegberg, *et al.* in 1951[4] and was introduced in the market in 1956. Cygon, Rogor, Dimethoate, Perfekthion, etc. are its common trade names. Dimethoate formulations are widely used as a contact and systemic pesticides against a broad range of insects and mites on numerous crops, fruits and vegetables. It is also used for the indoor control of houseflies. Heavy contamination of water bodies by these pesticides can lead to mass mortality of fish and other aquatic fauna.

The common carp, *Cyprinus carpio communis* is a highly palatable fish and is preferred for culture due to its high growth rate and prolific breeding in confined waters.

The aim of the current study was the estimation of acute toxicity of different concentrations of Dimethoate to common carp, *Cyprinus carpio communis* which is a recommended fish species for bioassay experiments and abundantly used as a food source in Kashmir (India). On the basis of this study we can compare toxicity of Dimethoate to other pesticides and can also use this fish as a model for other fish species. The reported results would be a useful contribution in the ecotoxicity risk assessments studies of Dimethoate on the fish species.

MATERIALS AND METHODS

Acetylcholinestrease (AChE) activity is a well established biomarker of exposure to organophosphate compounds in fish. It is present in cholinergic nerves and is responsible for the degradation of the neurotransmitter acetylcholine. Organophosphate binds irreversibly to the esteric site of AChE and thereby rapidly inhibits its activity. As AChE activity has a very high sensitivity to organophosphrous compounds, it has been considered to be a specific biomarker for these (Thomas, 1990)[5].

The brain of the fish was selected for assay of cholinesterase (AChE) since it provided a relatively large amount of the specific AChE in a readily accessible tissue (Mendal and Rudney, 1943)[6].

Live-healthy specimens of *Cyprinus carpio communis* were obtained from Hazratbal Market, Kashmir (India) and brought to the laboratory in plastic buckets with sufficient air. The plastic buckets were opened and the fish specimens were shifted to the glass aquaria (60 liter capacity) for about three weeks to be acclimatized to laboratory conditions and to eliminate transport-induced stress and allow for capture induced mortalities prior to pesticide exposure. During acclimatization periods fish were feed daily with commercial fish feed. Leftover food and excreta was removed daily when water of the aquaria was changed. Dead fish, whenever located were removed immediately to avoid fouling of the test medium.

The specimens were about 09 ± 1.05 cm in length and 50±1.02 gm in weight. New supplies of fish were obtained occasionally so that the fish material was seldom kept in the laboratory aquaria for the long duration.

The fish were divided into four groups (7 in each) and kept in 36 liter glass aquaria containing chlorine free tap water of pH 7.2; hardness, 154 mg/l (as CaCo3); dissolved oxygen, 7.4 mg/l (APHA)[7]. The untreated group-I served as control. The group-II, group-III and group-IV were exposed to a toxicologically safe concentration 0.80, 1.15 and 1.45 mg/l respectively of commercial formulation of Dimethoate. The highest concentration of pesticide which does not produce any apparent harmful effect in 96 hour of exposure has been termed the toxicologically safe concentration (Mouat and Stephan, 1967)[8].

The fish brains were recurred by cutting off the top of the skull and snipping the brain loose at the optic nerves and base of the medulla. This permitted lifting the brain free of the skull; the wet weights of the brains were determined by weighing on small pieces of tarred aluminum foil on a sensitive balance. The weighed brain was then transferred to a tissue solutions of the following composition; 0.2M NaCl, 0.02M $MgCl_2$, 0.194M K_2HPO4 and 0.006M KH_2PO4 with the pH adjusted to 8.2.

After draining and rinsing from homogenizer, the brie was diluted with additional buffer to contain approximately 1-4 mg of brain tissue per ml of dilution.

One milliliter of the diluted brain brie was pipetted into a test tube and incubated for 20 minutes at 25^0C with 1ml of 0.004 acetylcholine iodide (sigma chemicals) prepared in 0.001M sodium acetate of pH 4.5.

For each fish (7 from each group), whole brain AChE activity was arrayed in triplicate according to procedure given by Ellman *et al*., 1961[9]. The activity was measured by following the increase of yellow color produced when the thioanion produced by the enzymatic hydrolysis of substrate (acetylcholine iodide) reacts with DTNB (5, 5`-dithiobisnitrobenzoate). Arrays were conducted at 25^0C change in absorbance at 405 nm was measured at 30-80 intervals for 2-3 minutes using a Gilfor spectrophotometer. Acetylcholinestrease activities were expressed as µmol acetylcholineiodide hydrolyzed/min/g of tissue.

RESULTS AND DISCUSSION

The AChE activity in brain of the experimental animal *Cyprinus carpio communis* was found to be inhibited at different rates in organophosphate treated fishes. The rate of inhibition of this enzyme at a given dose and duration of treatment was more in increased concentration of the test substance (Dimethoate). Acetylcholine esterase activity in the brain of Dimethoate treated *Cyprinus carpio communis* was observed significantly inhibited as 20%, 29% and 35% for Dimethoate concentrations of 0.80 mg/l, 1.15 mg/l and 1.45 mg/l respectively for 96 hours (Fig 1).

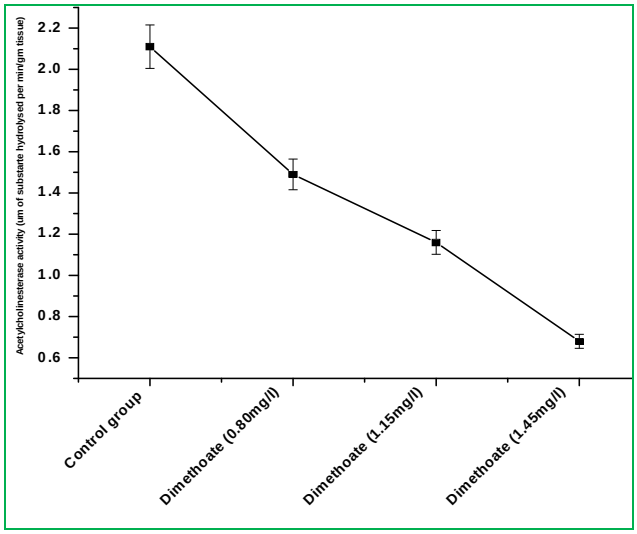

Figure 1: Schematic representation of the quantitative values (mean±SD in graphs) of AChE activity in the brain of female common carp after exposure of different concentrations of Dimethoate organophosphate pesticides for 96hr.

Organophosphate pesticides are competitive inhibitors of acetylcholinestrease (AChE), the key enzyme in the transmission of nerve impulse. AChE is readily phosphorylated by the organophosphate pesticides at the active site serine (Aldrige and Reiner, 1972[10] and Taylor, 1990[11]). The selectivity of action of organophosphates is that it causes inhibition of AChE and accumulation of acetylcholine at the synapse over stimulating the postsynaptic cells (Loskowsky and Dettbam, 1975[12] and Pope *et al*., 1995[13]). Reports also demonstrated that the organophosphate pesticide agents can bind to the acetylcholine receptors and this direct interaction is responsible for the manifestation of stress (Pope *et al*., 1995)[13]. Therefore, the AChE activity in tissues (Brain) in experimental animal *Cyprinus carpio communis* has been used in the present investigation as the neurophysiologic marker. Several authors have reported that enzymes of the same tissue of different species show difference in the sensitivity to various organophosphate insecticides (Pan and Dutta, 1998[14] and Monserret and Bianchini, 1998)[15]. In the present work all the groups treated with Dimethoate organophosphate pesticide revealed significant (P>0.01) inhibition of AChE activity in the brain of exposed fish. The inhibition of enzyme was more significant at higher doses of pesticides to fish in both the

cases. The time, dose and species related differences in enzyme susceptibility to organophosphate pesticides can primarily be attributed to dissimilar enzyme amount and inhibitor affinity degree to cholinesterase receptor. Although 50% or more depletion is supposed to be life threatening, available investigation shows that some fish are capable to tolerate over 90% inhibition in AChE activity (Day and Scott, 1990)[16]. More than 90% depletion was also reported in fish exposed to various insecticides (Balint *et al*. 1995[17] and Pan and Dutta, 1998[14]). The highest reduction in the present study was 72% which is considerably low. Oruce and Usta, 2007[18] reported that *Cyprinus carpio* showed more resistant to diazinon, this may be because of its low rate of bioactivation and relatively high activity of detoxicating enzymes (Keizer *et al*., 1991)[19]. Rath and Misra, 1981[20] and Ansari and Kumar, 1984[21] reported that inhibition of acetylcholine activity has relation with age of fish, concentration of pesticide and time of exposure. Their findings extended a considerable support to our observations.

CONCLUSION

The present study demonstrates that the organophosphate pesticides (Dimethoate) are potent inhibitors of brain AChE activity, but under identical dose the rate of enzyme inhibition was different for different pesticides.

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