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# Effect of an Organophosphate Insecticide Diazinon on the Activity of Acetylcholinesterase and Lipid Peroxidation of a Common Carp, *Cyprinus Carpio* L.

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**Abstract.-** Diazinon is a widely used organophosphorus pesticide in agriculture and environmental health, hence its adverse effects on non target animals, especially on fish is to be determined. The present research is conducted to study the influence of diazinon [0, 0-diethyl 0-(2-isopropyl-6-methylpyrimidin-4yl) phosphorothioate] on acetylcholinestrase (AChE) and lipidperoxidation (LPO) on freshwater common carp (*Cyprinus carpio* L.). Adult fish (mean body weight 42.6±3.86 g and 19.1±2.66 cm mean length ) were divided into five groups with 3 replicates and subjected to 5 concentrations (100, 200, 300, 400 and 500 µg $L^{-1}$) of diazinon for 30 days and compared with control (untreated) fish. Fishes were acclimatized to laboratory conditions for 7 days and then Physio-chemical characteristic of water was determined before the treatment. Induction of oxidative stress in various tissues was evidence of increased lipid peroxidation levels seems to be associated with the concentration of diazinon. Acetylcholinesterase activity responded positively in a concentration dependent pattern. It was concluded that LPO and AChE could be a valuable biomarker as an indicator of water pollution on aquatic ecosystems.

**Key words:** *Diazinon*, acetylcholinesterase, lipid peroxidation, stress, biomarkers, pollution.

## INTRODUCTION

Diazinon is one of the most frequently used pesticides in the agricultural region worldwide. It is agriculturally and commercially used to control adult and juvenile forms of insects in soil, plants, fruit, and crops. It is also used to control a variety of insects in a household environment (Cox, 1992). In addition, it is one of the main chemicals used as a biocide to suppress the excessive propagation of *Daphnia* zooplankton in fish farming (Machova *et al*., 2007). It is a common practice for fish farmers to use chemotherapeutants which have organophos-pates (like diazinon) among their active gradients against fish parasites in the aquaculture industry, which increases the risk of intoxication in cultivated fish species (Tryfonos *et al*., 2009). Diazinon, on the other hand, has been proven to be of great concern because it is toxic to fish at environmentally relevant concentrations (Couillard *et al*., 2008). Detection of biochemical changes caused by pesticides in fish development, therefore, is essential to assess the adverse effects of pesticides on fish, and to predict their impact on survival and growth (Oruc, 2010; Nuwani *et al*., 2013).

In the recent decades, there has been a growing concern for the protection of aquatic ecosystems against the adverse effects of contaminants, as a consequence of anthropogenic activity (Oropesa *et al*., 2009). Diazinon is easily washed into surface waters and enters the groundwater, which may affect a wide range of nontarget organisms including fish (Oruc, 2010). Among the potentials, mechanisms of organophosphate insecticide (OPI) toxicity are the induction of oxidative stress. Oxidative stress is able to compromise many vital functions and lipid peroxidation (LPO) is a major mechanism reported to be involved in the oxidative cell injury (Bassi *et al*., 2000). LPO is a complex process in biological membranes which are rich in polyunsaturated fatty acids. Lipid hydroperoxides decomposing double bonds of unsaturated fatty acids and destructing membrane lipids causing LPO (Ribera *et al*., 1991). Some studies reported that OPIs caused LPO (Kalender *et al*., 2007) in vertebrates. OPIs are shown to exert their action by inhibiting activity of acetyl cholinesterase (AChE) which plays an important role in neurotransmission at cholinergic

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synapses by rapid hydrolysis of the neurotransmitter acetylcholine to choline and acetate (Kwong, 2002) resulting in accumulation of acetylcholine (Fulton and Key, 2001). This leads to tremors, convulsions and finally the death of the aquatic organism. Several factors seem to be involved in affecting the AChE activity caused by OPIs such as length of time and exposure concentration. The toxicity of diazinon depends on the inhibition of AChE activity (AChE, EC 3.1.1.7) like other OPIs (Chambers and Carr, 1995). Therefore, measurement of AChE activity in the fish has been described as a method for diagnosing anticholinesterase pesticides in aquatic solutions (Archana *et al*., 2011). The knowledge of the major factors responsible for the species selective toxicity of this compound among fish may help to improve the classification of OP compounds

Therefore, the present study was aimed to assess the effect of the sublethal concentration of Diazinon on AChE and LPO activity in vital tissues of *C. carpio,* as biomarker of exposure to pollutants and to study their potential interest in predicting their toxicity.

## MATERIALS AND METHODS

*Collection and maintenance of fish*

Healthy and active *Cyprinus carpio* fish was purchased from the Fish Farm maintained by the Fisheries Department in the Kingdom. Fingerlings of common carp (*Cyprinus carpio* L.) with body weight 42.6±3.86 g and 19.1±2.66 cm mean body length were used for the experiment. Fish were brought to the laboratory in large aerated crates, acclimated for 30 days in large fiber tanks (22 **x** 12 **x** 5 feet) and fed with commercial dry feed pellets.

In the laboratory, the fish were held in 180 L glass aquaria (120 cm **x** 45 cm **x** 80 cm) containing dechlorinated tap water for acclimatization (20 days) at 25±1°C. The physico-chemical characteristics of the tap water were analyzed following the methods mentioned in APHA (1998). Water was renewed every day and a 12-12 h photoperiod was maintained during acclimatization and experimental periods. The fish were fed regularly with commercial fish food pellets during acclimatization and test tenures.

*Experimental design*

Sublethal concentrations of diazinon for the assay (50, 100, 200, 300 and 400 µg/L) were used, such concentrations were determined by previous experiment. Stock solutions of the test substance were prepared by dissolving the insecticide in tap water. These solutions were further diluted to obtain the experimental concentrations in aquaria. Fish were divided into 18 experimental aquaria (180 L): one control group (n = 10) and five treated groups treated with Diazinon with three replicates per test concentration were used to avoid test repetition due to system failure and to provide a stronger statistical baseline. The control group was provided with the same condition but without any Diazinon. Aeration was constant during the experimental period. The fish were starved for 24 h prior to experimentation to avoid prandial effects and to prevent the deposition of feces in the course of the assay. Dissolved oxygen, temperature, conductivity and ammonia were monitored regularly. During this period, sub-lethal endpoints such as level of activity, swimming performance and color changes were monitored. The exposure period lasted for 30 days during which the exposure media were renewed daily. AChE and LPO and were used as important biomarkers for detection of toxic nature of this pesticide. AChE was evaluated to determine the level of neurotoxicity whereas LPO was evaluated in terms of MDA for oxidative stress.

*Biochemical estimations*

*Tissue homogenate preparation*

At the end of the experiment, fish from control as well as treatment groups were removed from the aquarium and tissue homogenate was prepared by following the method described by Ghazala (2013). Enzyme activities were determined by using a spectrophotometer (Hitachi).

*Measuring AChE and LPO*

AChE activity is measured according to the method suggested by Ellman *et al*. (1961). The thiobarbituric acid reactive substances (TBARS) assay was used to evaluate the LPO according to Esterbauer and Cheeseman (1990).

*Protein determination*

The protein concentrations of the brain,

muscle and gill samples were measured using the Lowry's method (Lowry *et al*., 1951), with bovine serum albumin as the standard. The enzymatic activity was calculated in terms of the protein content of the sample.

*Statistical analysis*

The data thus obtained was subjected for appropriate statistical analysis experiments by one-way ANOVA analysis ($p<0.05$), followed by Tukey's test (means comparison) using a statistical software SPSS 18.0. Statistical comparisons were done between control and treated groups of *C. carpio*.

## RESULTS

The temperature ranged from 34 to 39°C during experimentation. The pH of the water ranged from 7.8 to 8.1, which was slightly higher than neutral. Dissolved oxygen ranged from 7.2 to 8.3 mg/L.

**Table I.- Acetylychholineesterase (AChE) in brain, gills and muscles of control and Diazinon treated *C. carpio*.**

| | Tissues (µg) | | |
|---|---|---|---|
| | **Brain** | **Gills** | **Muscles** |
| Control | 1.62 ± 0.02 | 0.95 ± 0.01[a] | 1.39 ± 0.04[a] |
| 50 | 0.92 ± 0.03[b] | 0.75 ± 0.04[b] | 1.17 ± 0.05[b] |
| 100 | 0.75 ± 0.01[c] | 0.69 ± 0.03 | 0.94 ± 0.04[c] |
| 200 | 0.60 ± 0.03[d] | 0.56 ± 0.02[d] | 0.79 ± 0.03[d] |
| 300 | 0.48 ± 0.01[e] | 0.49 ± 0.02[e] | 0.57 ± 0.01[e] |
| 400 | 0.39 ± 0.02[e] | 0.43 ± 0.01[e] | 0.49 ± 0.02[f] |

The values are expressed as Means ± SE (n=10). LPO activity expressed as nanomoles of MDA formed/mg protein. The different superscriptd in the same row indicated significant differences in treatments ($p\leq 0.05$)

*Anticholinesterase potential of diazinon*

The results showed that diazinon has a general anticholinesterase potential. AChE activity measurement in different diazinon concentrations (50, 100, 200, 300 and 400 µg $L^{-1}$) is presented in Table I. Exposure of diazinon to various doses depicted a general dose-dependent inhibition of AchE in brain, muscle and gills of *C. carpio* compared with control in all experimental doses. The comparison of means for brain and gills and muscles showed significant ($P<0.05$) differences for the inhibition of AchE among all the treatments. It has been observed that the inhibition in AchE was correlated with the concentration of the diazinon. In muscle and gills there is a general inhibition in AchE activity but these values are found to be not significant. Among the three tissues, the maximum inhibition in the activity of AchE was found in the fish brain.

*Levels of lipid peroxidation*

The results indicated that diazinon caused a significant increase in LPO values. LPO activity measurement in the presence of different diazinon concentrations (50, 100, 200, 300 and 400 µg) $L^{-1}$ is presented in Table II. Exposure of diazinon resulted in a significant induction of LPO in brain, muscle and gills of fish compared with control. An increase in the mean values of MDA (Melanoaldehyde) for 100 µg $L^{-1}$ exposure was significant at in brain, liver and gills (Table II). It has been observed that LPO level increased significantly with an increase in the concentration of diazinon (Table II). The highest mean values of MDA of LPO were measured in all organs at 400 µg $L^{-1}$ exposures. The comparison of the means indicated that the differences between the various concentration significant ($p<0.05$). Among the three tissues, the maximum values of MDA were recorded for muscles of *C. carpio*.

**Table II.- Lipid peroxidation (LPO) in brain, gills and muscles of control and Diazinon treated *C. carpio***

| | Tissues (µg) | | |
|---|---|---|---|
| | **Brain** | **Gills** | **Muscles** |
| Control | 0.42 ± 0.01[f] | 0.32 ± 0.02[f] | 0.92 ± 0.05[f] |
| 50 | 0.67 ± 0.02[e] | 0.54 ± 0.03[e] | 1.03 ± 0.06[e] |
| 100 | 0.85 ± 0.03[d] | 0.77 ± 0.05[d] | 1.22 ± 0.04[d] |
| 200 | 1.10 ± 0.05[c] | 0.99 ± 0.04[c] | 1.55 ± 0.04[c] |
| 300 | 1.41 ± 0.06[b] | 1.31 ± 0.04[b] | 1.78 ± 0.05[b] |
| 400 | 1.58 ± 0.05[a] | 1.63 ± 0.06[a] | 2.34 ± 0.08[a] |

The values are expressed as Means ± SE (n=10). LPO activity expressed as nanomoles of MDA formed/mg protein. The different superscriptd in the same row indicated significant differences in treatments ($p\leq 0.05$)

## DISCUSSION

The results of the present study have demonstrated that the applied doses of diazinon could have affected the LPO and AChE level in fish. OP pesticides have several toxic properties, the most prominent effect of which is AChE inhibition.

AChE activity is therefore widely used in biomonitoring studies as a biomarker of OP pesticide exposure. In this study, the reduction of AChE activity is assumed to result from the direct action of diazinon exposure on the active site of this enzyme. All groups tested by diazinon in this study revealed an inhibition of AChE activity in all treated organs of *C. carpio*. These findings were in accordance with the results of Rath and Misra (1981). They reported a positive correlation with insecticide concentration and the time of exposure associated with the degree of AChE inhibition of *Tilapia mossambica* in relation to the interacting effects of sublethal concentrations of dichlorvos . Similar results have also been reported in sunfish, *Lepomis gibbosus* (Benke and Murphy, 1974); in *Danio rerio* (Ansari and Kumar, 1984), *Seriola dumerilli* (Jebali *et al*., 2006), freshwater catfish *Heteropneutes fossilis* (Chandra, 2008) after malathion exposure and in *Poecilia reticulate (*Archana *et al*., 2011). A decrease in AChE activity by diazinon intoxication has been reported in different animals and fish as *Oreochromis niloticus* (Tridico *et al*., 2010). However, most of the AChE studies are done in fish brains, because the most pronounced effects are observed in nervous tissue. The brain of C. *carpio* exhibited the highest AChE inhibition. Rao and Rao (1984) compared the AChE inhibition in different tissues of the teleost, *Tilapia mossambica* exposed to 1/3 of methyl parathion $LC_{50}$ for 48 h. Afterwards, they observed that the brain had the highest inhibition levels followed by muscle, gills and liver. Ram *et al*. (2011) reported that Polytrin C which is a combination of pesticide significantly inhibits the plasma and brain AChE levels of Wistar rats. Rao *et al*. (2008) reported that seven different synthetic compounds of Imidacloprid with various substitutions caused a high concentration of AChE which may be due to its inhibitory action in post-synaptic regions of nerves. The AChE inhibition is fairly related to the tissue enervation level. Hence, it can be concluded that the highest AChE concentration the highest inhibition susceptibility. Specific AChE activity significantly ($P < 0.05$) decreased in *C. carpio* in all tissues, after the 30 days of experimental exposure to diazinon. OP pesticides have several toxic properties, the most prominent effect of which is AChE inhibition. AChE activity is, therefore, widely used in biomonitoring studies as a biomarker of OP pesticide exposure. In this study, the reduction of AChE activity is assumed to result from the direct action of diazinon exposure on the active site of this enzyme.

The extent of LPO is determined by the balance between the production of oxidants and the removal and scavenging of those oxidants by antioxidants (Filho, 1996). Generation of oxidative stress and consequent LPO by pesticides is reported in many species. Due to high concentration of polyunsaturated fatty acids in cells, LPO is a major outcome of the free radical mediated injury. Two broad outcomes of LPO are structural damage of cellular membranes and the generation of oxidized products, some of which are chemically reactive and may covalently modify cellular macromolecules. These reactive products are thought to be the major effects of tissue damage from LPO (Mattson, 1998). One of the most damaging effects of free radicals and their products in cells is the peroxidation of membrane lipids of which MDA is an indicator. MDA is the final product of LPO and a sensitive diagnostic index of oxidative injury in cells. LPO is one of the major mechanisms involved in the oxidative cell injury (Bassi *et al*., 2001). MDA is a major oxidation product of peroxidized polyunsaturated fatty acids and increased MDA content is an important indicator of LPO. However, when a low level of stress is applied, an adaptive response takes place in the cells. This adaptation may be associated with de novo protein synthesis, or might be due to the activities of various removal and repair enzymes. In the present study, an increase was found in MDA content, while protein content decreased after 15 days of diazinon exposure. Diazinon can inhibit protein synthesis or induce metabolic pathways leading to protein reduction (Handy *et al*., 2002). Deficiency in protein content may be related to general stress response. Fish, under stressful conditions, secrete high amounts of catecholamine which deplete glycogen reserves and use proteins as an energy source for muscle. Detailed studies have provided evidence that many species exhibit an increased MDA following stress produced by some xenobiotics (Luo *et al*., 2005). However, when a low level of stress is applied, an

adaptive response takes place in the cells. This adaptation may be associated with de novo protein synthesis, or might be due to the activities of various damage removal and repair enzymes. Ince *et al*. (2010) reported that dorsal skin application of deltamethrin (7.5 g $L^{-1}$) for 7 days significantly increased blood MDA level. Salama *et al*. (2005) reported that upon 48 h exposure to various pesticides to land snail *Helix aspersa* carbafuran significantly inhibited the AChE levels while none of the pesticide was found to induce LPO level. The results of the present study have demonstrated that the applied dosages of diazinon could have affected the AChE and MDA concentration in the fish. This is evidenced from our findings that, upon diazinon treatment in vivo, the concentration of AChE and MDA in brain, muscle and gills differs from those of controls. MDA is a major oxidation product of and an increased MDA content is an important indicator of LPO (Archana *et al.*, 2011). The increased MDA content might have resulted from an increase of free radicals as a result of stress condition in *C. carpio* with diazinon intoxication. The results of the present study showed that MDA content significantly increased in the all tissues of fish treated with five different doses of pesticide. Similar findings were also reported in rainbow trout, *Oncorhynchus mykiss* (Isik and Celik, 2008) and in *C. carpio* (Oruc, 2010). The present findings revealed that diazinon not only changes AChE activity but also affects LPO. The findings also indicated that *C. carpio* has a good antioxidant defense system which can reduce oxidative damages.

## CONCLUSION

The present investigation indicated that the pesticide diazinon is a toxin to common carp. We further concluded from our observations the concentration of AChE and MDA in brain, muscles and gills differs from those of controls upon treatment with diazinon. It is proposed that AChE and LPO in fish could be effectively used as biomarkers of pesticide toxicity.

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