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Effect of Organophosphate Insecticide on the Organic Constituents in Liver of *Channa punctatus*

D. P. Jaroli* and B. L. Sharma
Department of Zoology
University of Rajasthan
Jaipur-302004, India

Abstract : Effect of Chlorpyrifos (Dursban) an organophosphate insecticide on the glycogen, cholesterol, protein, alkaline phosphatase and acid phosphatase in liver in *Channa punctatus* (Bloch) exhibited notable alterations. Liver being the main site of metabolic activity in body and highly active in both toxifying and detoxifying insecticides, was selected for the study purpose. Chlorpyrifos insecticide caused significant increase in the activity of alkaline phosphatase, acid phosphatase and cholesterol while decrease in glycogen and protein contents in the liver of treated fish. The results related to regressive phenomenon with in the cells. It is found that Dursban disturb the chemical constituents of the fish which leads to cell damages and finally death of fishes.

Key words : Chlorpyrifos (Dursban), liver, tissue biochimestry, *channa punctatus.*

Introduction :

The aquatic life is constantly threatened by the seepage of pesticides and their constituents from agricultural fields, industrial and domestic sewage. Fishes are much vulnerable to toxic substances and their bioaccumulation cause serious risk to life. Such toxic substances enter to humen through food chain, as fishes constitute an important part of animal protein in rural and urban areas. Their availability and selectivity to toxic substances are main criteria for selection as an experimental animal.

The mechanism by which the organophosphorous insecticides exert their toxic action on the arthropods or fishes depends largely on the biochemical processes of animal and the physico-chemical properties of phosphorous compounds. Fundamentally, however, the toxic symptoms produced in animals by these organophosphorous compounds are manifestations of the inhibition of certain enzyme systems. Organophosphorous insecticides are inhibitors of variety of estrases, but generally they are associated with inhibition of cholinestrase (Ravi and Selvarajan, 1990).

* Corresponding Author

The intake of insecticides affects the biochemical composition of fishes (Jebakumar *et al.,* 1990; Sultatos, 1998; Kumble and Muley, 2000; Prasad *et al.*, 2002). It has been shown by many scientists that insecticides mainly affect liver of fishes (Murty and Devi, 1982; Anthony *et al.,* 1986; Bhushan *et al.,* 2002). This is because of its relatively slow blood flow as compared to cardiac output (Gingerich, 1982) as well as the much closer association of hepatocytes to biliary system than is found in mammals (Hinton and Lauren, 1990).

Dursban, an organophosphate insecticide that has been in use around for more than 35 years, is used in present investigation. The technical name of chemical is chlorpyrifos. It is the most widely used insecticide in India as well as in other parts of the world. It is a relative of nerve gases developed by Nazis during World War II. Originally it was used primarily to kill mosquitoes, but now it is no longer registered for this use. Chlorpyrifos is being widely used against cutworms, corn rootworms, cockroaches, grubs, flea beetles, flies, termites, fire ants and lice. It is used as an insecticide on grains, cotton fields, fruits, nut and vegetable crops and as well as on lawns and ornamental plants. Pesticide ingredient chlorpyrifos is known as Dursban in non-agricultural products, while in agriculture it is called Lorsban. For simplicity, we refer to chlorpyrifos in all cases as Dursban. When it is used against termites, it is known as termicide and when it is used to kill insects it is termed as insecticide. The present piece of work includes a detailed account of sublethal effects of insecticides on the liver of fresh water, air-breathing, Indian murrel, *Channa punctatus* (Bloch), a commercially important fish. Liver being the main site of metabolic activity in body and highly active in both toxifying and detoxifying insecticides, was selected for the study purpose.

Materials and Methods :

Channa punctatus (Bloch) is a cosmopolitan fresh water teleost, which is found in abundance in the lake waters of Rajasthan. This species is commonly known as murrel. It is carnivorous, mud dweller and air breathing fish. It feeds on small fishes and insects. The fishes of average length (15-18 cm) and weight (26-30 g) were collected from Jal Mahal and Ramgarh Lakes, situated in the vicinity of Jaipur (India). They were

acclimated for 15 days to aquaria conditions, containing water with stable physico-chemical properties. The fishes were fed with minced goat liver twice a week. Static bioassay test of water was done according to "Standard methods for the examination of water and waste water" (APHA *et al.*, 1995).

For the determination of LC_{50} concentration, five groups of 10 acclimated fishes were taken in each glass aquarium of capacity, 50 liters. Each group was exposed to 0.037, 0.075, 0.15, 0.30 and 0.60 ppm of Dursban. Water and dose were replaced daily. Mortality of fishes was recorded in each group for 96 h. The regression equations were established by using probit - mortality and log of concentration of pesticide and LC_{50} value was determined. The three sublethal concentrations, 10%, 20% and 30% of LC_{50} was calculated for experimental studies.

Four aquaria, each filled with 50 liters of water and 20 acclimated fishes, were taken. The four groups of fishes were treated for 15 days as follow :

Group I : Control

Group II : Exposed to 10% of LC_{50} value

Group III : Exposed to 20% of LC_{50} value

Group IV : Exposed to 30% of LC_{50} value

To study the effects of chlorpyrifos, five fishes from each group were sacrificed by cephalic stunning and autopsied, at the end of 1, 3, 7 and 15 days of exposure. The liver was dissected out, freed off excess tissues and processed for tissue biochemical studies.

In the liver of *Channa punctatus* glycogen (Montgomery, 1957); cholesterol (Zlatkis *et al.*,1953); protein (Lowry *et al.*, 1951); alkaline phosphatase (Fiske and Subbarow, 1925) and acid phosphatase (Fiske and Subbarow, 1925) were estimated quantitatively, as per the methods by workers in parenthases, respectively.

Observations and Results :

Mortality studies showed that the sublethal level, LC_{50} of *Channa punctatus* (Bloch) for 96 h exposure was 0.365 ppm for Dursban. The

minimum effective doses 10% of LC_{50} (0.037 ppm), 20% of LC_{50} (0.075 ppm) and 30% of LC_{50} (0.110 ppm) were calculated for experimental purposes. Biochemical estimations of glycogen, cholesterol, protein, alkaline and acid phosphatase in liver of control as well in treated *Channa punctatu*s were carried out. The results are as follows (Table 1) :

Table 1 : Changes in certain metabolites and enzymes in liver of *Channa punctatus* (Bloch) during control and post-treatment with Dursban insecticide

Groups	Parameters				
	Glycogen (mg/g)	**Cholesterol** (mg/g)	**Protein** (mg/g)	**AlkPase** (mg pi/g/h)	**AcPase** (mg pi/g/h)
I (Control)	3.42±0.08	18.46±0.48	161.12±5.56	11.02 ± 0.34	6.52 ± 0.11
II (10% of LC_{50})	2.28±0.14^{z} (33.33)	23.05±0.47^{z} (24.86)	93.28±4.48^{z} (42.11)	12.79 ± 0.19 ^{y} (16.06)	10.68 ± 0.41^{Z} (63.80)
III (20% of LC_{50})	2.29±0.14^{z} (33.04)	26.89±0.32^{z} (45.67)	106.54±4.78^{z} (33.88)	14.12 ± 0.17 ^{z} (28.13)	12.47 ± 0.21^{Z} (91.26)
IV (30% of LC_{50})	2.14±0.14^{Z} (37.43)	28.23±0.25^{z} (52.93)	115.54±4.89^{z} (28.29)	15.69 ± 0.49 ^{z} (42.38)	13.23 ± 0.07^{Z} (102.91)

LC_{50} value : 0.365 ppm

Values are expressed mean ± SE of five observations

Values are significant at $^{x}P<0.05$, $^{y}P<0.01$, $^{z}P<0.001$

Values in the parentheses indicate % stimulation

Glycogen : The quantity of glycogen in the liver of the normal fish was 3.42 mg/g. At the end of 15 days of exposure to 0.037 ppm, 0.075 ppm and 0.110 ppm the quantity came down to 2.28 mg/g, 2.29 mg/g and 2.14 mg/g, respectively.

Cholesterol : The quantity of cholesterol in the liver of the normal fish was 18.46 mg/g. When exposed to 0.037 ppm, 0.075 ppm and 0.110 ppm the quantity of cholesterol rise to 23.05 mg/g, 26.89 mg/g and 28.23 mg/g, respectively.

Protein : The protein content of the normal fish was 161.12 mg/g. When exposed to 0.037 ppm, 0.075 ppm and 0.110 ppm of chlorpyrifos, the protein content reduced to 93.28 mg/g, 106.54 mg/g and 115.54 mg/g, respectively.

Alkaline phosphatase : The alkaline phosphatase activity was 11.02 mg pi/g/h at 37º in the liver of normal fish. With the exposure to 0.037 ppm, 0.075 ppm and 0.110 ppm of chlorpyrifos, the increased enzyme activity were 12.89 mg pi/g/h, 14.12 mg pi/g/h and 15.89 mg pi/g/h, respectively.

Acid phosphatase : The activity was 6.52 mg pi/g/h at 37º in the liver of normal fish. With the exposure to 0.037 ppm, 0.075 ppm and 0.110 ppm of chlorpyrifos, the increased enzyme activity measured were 10.68 mg pi/g/h, 12.47 mg pi/g/h and 13.23 mg pi/g/h, respectively.

Discussion :

The fishes were subjected to different doses of insecticide and biochemical estimation of glycogen, cholesterol, protein, alkaline phosphatase and acid phosphatase in liver was studied.

Glycogen : The toxic effects of parathion an organophosphate pesticide has been a decline in the liver glycogen due to depression of glucokinase activity resulting in the reduction of glucose 6-phosphate (Piccaluga *et al.*, 1967). Possibly this very mechanism brought about a reduction of glycogen in *Channa punctatus* also. Whereas Mishra and Srivastava (1983) observed no changes in liver glycogen content in malathion treated catfish *Heteropneustes fossilis*. Possibility for reduction in the glycogen level may be due to destruction of glucose-6-phosphatase, which is located in the membrane of endoplasmic resticulum as a consequence of Dursban administration. Similar observation has been made by (Hurkat, 1978) in dieldrin intoxicated rabbits. A close relationship between the smooth membrane and glycogen inclusion in normal hepatocytes (Millonig and Porter, 1961). The histological damage to hepatocytes and disappearance of glucose-6-phosphate by Dursban may be a possible cause for decrease in glycogen content of *Channa punctatus*.

Cholesterol : Under the influnce of any pescides, liver experiences stress, metabolically. In such a situation the excretory mechanism is disturbed and leads to an increases.

Protein : The reduction in protein content may be due to increased utilization of protein to meet out the energy demand when the fish is under stress condition. Rath and Mishra (1980) have also reported such reduction in protein content when the fish were exposed to pesticide media.

Decline in the protein content of liver would suggest an intensive proteolysis which in turn contributes the increase of free amino acids to be fed into the TCA cycle as keto acids, thus supporting the view of Kabeer *et al.* (1978).

Alkaline phosphatase : Functional importance of alkaline phosphatase in the animals has been demonstrated by many workers. The enzyme is associated with glycogen and is linked with transportation of intermediate compounds in glycogenesis or glycogenolysis but it has also been linked with DNA in the nucleus, secretion and formation of fibrous proteins (Bradfield, 1950) and tissue growth (Malone, 1960).

Our results are in corroboration with the results of others who have also reported increased alkaline phosphatase activities in the liver which has been reported to be induced by the action of varieties of chemical agents including pesticides. The increase in the liver alkaline phosphatase induced by different insecticides has also been reported by many workers (Joshi and Desai, 1981; Kumar and Kumar, 1997; Singh and Singh, 2002). Elevation in alkaline phosphatase activity might be due to an accelerated membrane transport function related to anion hydroxide exchange across the lipid biomembranes mediated by organotin compounds. Another possibility for the increase in the activity of alkaline phosphatase may be the destruction of the hepatic smooth endoplasmic reticulum membrane (Khan and Pandya, 1985) in the insecticide intoxicated mice as supported by pathological observations made in the hepatic tissue of intoxicated fish. This increase in the hepatic alkaline phosphatase activity was due to the cellular damages caused by hepatotoxins or a response to overcome toxicity of Dursban.

Acid phosphatase : Acid phosphatases are hydrolytic lysosomal enzymes and are released by the lysosomes for the hydrolysis of foreign

material, hence it has a role in certain detoxification functions. It is known as inducible enzyme whose activity in animal tissue goes up when there is a toxic impact and the enzyme being to counteract. Subsequently the enzyme activity may begin to drop either as a result of having partly or fully encountered the toxin or as a result of cell damage.The increase in hepatic acid phosphatase activity in intoxicated animals as observed in the present investigation may be due to the destruction of the lysosomal membrane which resulted in the release of the enzyme.

In the present study, the acid phosphatase activity of liver of *Channa punctatus* increased in all the three doses. Such increase is also found as a function of exposure period. The pronounced increase in protease activity may be due to the damage caused to the lysosomal membrane, thus permitting the leakage of lysosomal enzyme into cytosol as suggested by Sherekar and Kulkurni (1987).

It is found that Dursban disturb the chemical constituents of the fish which leads to cell damages and finally death of fishes.

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