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Effect of Endosulfan on Acid and Alkaline Phosphatase Activity in Liver, Kidney, and Muscles of *Channa gachua*

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The widespread use of a great many toxic chemicals to eliminate unwanted plant or animal species has resulted in the contamination of most aquatic habitats with these substances on a regular basis. Pesticides enter into the hydrosphere via many pathways including: direct application for pest and disease vector control, urban and industrial waste-water discharges, surface runoff from nonpoint sources including agricultural soil, aerosol and particulate deposition, rainfall, and absorption from the vapor phase at the air-water interphase etc.

Endosulfan, a polycyclic chlorinated hydrocarbon of cyclodien group, is a well known organochlorine insecticide. WHO (1984) classified endosulfan in the category of technical products that are moderately hazardous. This study reports the effect of endosulfan (6, 7, 8, 9, 10, 10-hexachloro -5, 5a, 6, 9, 9a-hexahydro -6, 9, -methano -2, 4, 3 - benzo dioxathiepin 3-oxide) on the activity of acid and alkaline phosphatase in liver, kidney and muscles of a freshwater teleost, Channa gachua.

MATERIALS AND METHODS

Living specimens of Channa gachua were collected from local freshwater sources and maintained in laboratory glass aquaria. Prior to experimentation, fish were allowed to acclimatize to the laboratory conditions for 10 days at 22 ± 1°C in tap water. The fish were fed with commercial fish food once a day. Some of the chemical characteristics of the water used are given in Table 1. Fish weighing 22 ± 5 g were selected and divided into three groups of 20 fish each. They were kept in 25-L experimental aquaria containing tap water.

The endosulfan stock solution of 1g/L of water was prepared. Aliquots of this stock solution were added to each experimental aquaria to bring the endosulfan concentration of 0.0022,0.0037 and 0.0056 mg/L. Another group maintained in tap water served as control. The water was changed

daily to reduce the build up of metabolic wastes and to keep concentrations of endosulfan near the nominal level. No fish moratality was observed during exposture period. After 15 and 30 days of exposure the animals were transferred to clean water, and liver, kidney, and muscles (red muscles from anterior region) of control and treated fish were removed.

Table 1. Chemical characteristics of the tap water used for experimentation. *All values except pH are in mg/L

Parameters	Values*
pH	7.4 - 7.6
Dissolved Oxygen	7.5 - 7.9
Total hardness (as Ca CO_3)	55.0 - 70.0
Total solids	30.4 - 33.5
Silica	2.1 - 2.2
Solids (Volatile)	11.1 - 13.1
Nitrogen (Organic)	0.11- 0.17
Chloride	1.2 - 3.1
Phosphate	0.1 - 0.5
Sulphate	4.1 - 4.5

For the estimation of enzyme activities, 10% (w/v) homogenates of liver, kidney and muscles were prepared in ice-cold 1.25 M sucrose solution. The homogenates were centrifuged for 20 min at 1500 g in a refrigerated centrifuge, and the clear supernatant fluids were used as the enzyme source. Sodium β -glycerophosphate (0.016 M) was used as substratae at pH 5.0 for acid phosphatase and at pH 9.3 for alkaline phosphatase. The enzyme activities were estimated by the method of Urrego and Epstein (1971) and are expressed as mg inorganic phosphate liberated/h/mg of protein. Protein in the homogenates was estimated by the method of Lowry et al (1951) and inorganic phosphate by the method of Fiske and Subba Rao (1925). The test described by Fisher (1950) was employed to calculate the statistical significance between control and experimental values.

RESULTS AND DISCUSSION

The acid and alkaline phosphatase activities in control and experimental fish are shown in Tables 2 and 3. Fish showed slight increase in acid phosphatase activity in liver and kidney after 15 days of exposure to 0.0022 mg/L endosulfan. But the enzyme activity in liver, kidney, and muscles was significantly inhibited at 0.0056 mg/L of endosulfan after chronic exposure of 15 days (Table 2). The maximum inhibition was observed in muscles (62.1%) followed by kidney (60.3%) and liver (55.2%). After 30 days of exposure at 0.0056

Table 2. Acid phosphatase activity in liver, kidney and liver of Channa gachua after 15 and 30 days of exposure to different concentrations of endosulfan. Values are average of five observations.

Time (days)	Concentration (mg/L)	Enzyme activity=mg of inorganic phosphate liberated/h/mg of protein Liver[a]	Kidney[a]	Muscles[a]
15	Control	0.0533 ± 0.0021	0.0256 ± 0.0013	0.0065 ± 0.0025
	0.0022	0.0553 ± 0.0028 (3.8)[b]	0.0269 ± 0.0017 (5.3)[b]	0.0319 ± 0.0020 (12.6)
	0.0037	0.0417 ± 0.0027 (21.6)*	0.0179 ± 0.0015 (30.2)*	0.0253 ± 0.0021(30.8)*
	0.0056	0.0239 ± 0.0024 (55.2)***	0.0102 ± 0.0010 (60.3)***	0.0138 ± 0.0024 (62.1)***
30	Control	0.0552 ± 0.0031	0.0265 ± 0.0011	0.0335 ± 0.0016
	0.0022	0.0410 ± 0.0029 (25.6)	0.0183 ± 0.0015 (30.9)*	0.0236 ± 0.0012 (29.5)**
	0.0037	0.0331 ± 0.0026 (39.9)**	0.0144 ± 0.0013 (45.6)**	0.0197 ± 0.0015 (41.2)**
	0.0056	0.0166 ± 0.0021 (69.9)***	0.0054 ± 0.0010 (79.6)***	0.0116 ± 0.0018 (65.5)***

[a]Mean ± SE; [b] percent stimulation; other values in parentheses are percent inhibition; values are significant at *P<0.05; **P<0.01; ***P<0.001 (Fisher's t test)

Table 3. Alkaline phosphatase activity in liver, kidney and liver of Channa gachua after 15 and 3o days of exposure to different concentrations of endosulfan. Values are average of five observations.

Time (days)	Concentration (mg/L)	Enzyme activity=mg of inorganic phosphate liberated/h/mg of protein Liver[a]	Kidney[a]	Muscles[a]
15	Control	0.0358 ± 0.0015	0.0278 ± 0.0018	0.0312 ± 0.0011
	0.0022	0.0379 ± 0.0012 (5.8)[b]	0.0262 ± 0.0017 (5.9)	0.0280 ± 0.0010 (10.1)
	0.0037	0.0266 ± 0.0010 (25.8)**	0.0213 ± 0.0016 (23.5)	0.0223 ± 0.0010 (28.6)**
	0.0056	0.0193 ± 0.0013 (45.9)**	0.0128 ± 0.0016 (53.8)**	0.0160 ± 0.0010(48.6)***
30	Control	0.0365 ± 0.0021	0.0255 ± 0.0011	0.0325 ± 0.0015
	0.0022	0.0271 ± 0.0021 (25.6)**	0.0178 ± 0.0016 (30.8)**	0.0246 ± 0.0013(24.2)**
	0.0037	0.0216 ± 0.0021 (40.8)**	0.0125 ± 0.0014 (50.7)**	0.0209 ± 0.0012 (35.6)**
	0.0056	0.0125 ± 0.0020 (65.8)**	0.0054 ± 0.0010 (78.5)***	0.0119 ± 0.0012(63.3)***

[a]Mean + SE; [b] percent stimulation; other values in parentheses are percent inhibition; values significant at **P<0.01; ***P<0.001 (Fisher's t test)

mg/L, the enzyme activity was further reduced and showed 65.5%, 69.9% and 79.6% lower values in muscles, liver, and kidney, respectivley. A similar pattern of effect on the alkaline phosphatase activity was observed at all the levels of endosulfan. The enzyme activity was found to be activated at 0.0022 mg/L after 15 days by 5.8% in liver (Table 3). However, at 0.0056 mg/L after the same duration of exposure time, liver enzyme activity decreased significantly ($P<0.01$). A gradual reduction in enzyme activity with increasing concentration and exposure time was observed. A significant inhibition ($P<0.01$) was observed in all the three tissues at both concentrations (0.0022 and 0.0037 mg/L) of edndosulfan after 30 days of exposure.

High dose of methyl benzimicazol carbamate (MBC) significantly ($P<0.01$) increased alkaline phosphatase activity in male rats (Janardhan et al 1987), which may be indicative of an adaptive rise in enzyme activity to the persistent stress (Murphy and Porter 1966). Sugawara and Sugawara (1975) reported a decrease in the alkaline phosphatase activity in the brush border of rat intestine and suggested that Cd decreases the absorption of Ca from intestine. Parathion, malathion, and phosalone caused significant reduction in hepatic alkaline and acid phosphatase activities in rats when fed orally for 21 consecutive days (Bulusu and Chakravarty 1987). Sastry and Gupta (1979) observed a significant reduction in enzyme activities in the liver and intestine of a freshwater fish, Heteropneustes fossils. Decreased alkaline phosphate and increased acid phosphatase activities in liver and kidney of Channa punctatus on exposure to endrin have been reported by Sastry and Sharma (1978). The decrease in alkaline phosphatase by pesticides probably indicates an altered transport of phosphate (Engstrom 1964) and an inhibitory effect on the cell growth and proliferation (Goldfischer et al 1964).

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