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Acid phosphatase activity in tissues of *Notopterus notopterus* chronically exposed to phenolic compounds

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Abstract. Specimens of *Notopterus notopterus* were exposed to three sublethal concentrations (1/10th, 1/15th and 1/20th of 96 hr LC_{50}) of phenol (P), 2,4-dinitrophenol (DNP), pentachlorophenol (PCP), and their three combinations (PCP + DNP)/P (highly antagonistic), (DNP + P)/PCP (additive) and (P + DNP)/PCP (highly synergistic) for 15 and 30 days, and brain, liver, kidney and gills were taken out separately for determining acid phosphatase activity. In general, inhibition was maximum (89·32%) and highly significant ($P < 0·001$) in brain, and minimum (6·93%) and insignificant in kidney of fish exposed to 1/10th of (P + DNP)/PCP and P, respectively after 30 days. When P, DNP and PCP were used separately PCP exerted more inhibitory effects than DNP and P. However, significant stimulation ($P < 0·05$; $P < 0·01$) at 1/15th and 1/20th of P and DNP both after 15 and 30 days, and insignificant at 1/20th of (PCP + DNP)/P after 15 days was also observed in kidney.

Keywords. Acid phosphatases ; *Notopterus notopterus*, phenolic compounds.

1. Introduction

There is increasing concern today about environmental contamination with phenolic compounds such as phenol, 2,4-dinitrophenol and pentachlorophenol. These compounds are the non-specific pesticides (Rappe and Nilson 1972) used as herbicides, molluscicides and bactericides in industries, wood preservation and agriculture. As an antiseptic, phenol is also used for medicinal purposes. Inspite of their extensive use, little attention is paid on their effects on metabolic activities of freshwater fish (Weinbach and Garbus 1969 ; Desaiah 1978 ; Dalela *et al* 1980 ; Verma *et al* 1980). Acid phosphatase is a hydrolytic enzyme which takes part in the dissolution of dead cells and as such is a good indicator of stress condition in the biological system (Gupta *et al* 1975 ; Verma *et al* 1980). This study was undertaken to evaluate the effects of sublethal concentrations of P, DNP, PCP and their three combinations—(PCP + DNP)/P (highly antagonistic), (DNP + P)/PCP (additive) and (P + DNP)/PCP (highly synergistic) (Verma *et al* 1981) on acid phosphatase activity (orthophosphoric monoester phosphohydrolase; E.C. 3·1·3·2) in different tissues of a freshwater fish *Notopterus notopterus*.

2. Materials and methods

Specimens of *N. notopterus* (16 to 21 cm in length ; 35 to 60 g in weight) were collected from Kalinadi and adopted for two weeks to the laboratory conditions. The technical grades of phenol (C_6H_5OH), 2,4-dinitrophenol ($(NO_2)_2 C_6H_3OH$) and pentachlorophenol (sodium salt; C_6Cl_5ONa) manufactured by Thomas Baker and Co. (London), Thomas and Thomas (India) and Hopkins and William Ltd. (England), respectively were used. Stock solutions of 1·0 g/L were prepared separately and the desired concentrations of these chemicals were obtained, using the table 231 (3) of Standard Methods (1971).

Fifteen fish were transferred in each concentration (1/10th, 1/15th and 1/20th of 96 hr LC_{50}) of these chemicals and combinations kept in triplicate for 30 days (96 hr LC_{50} of P, DNP, PCP and (PCP + DNP)/P, (DNP + P)/PCP and (P + DNP)/PCP combinations being 12·53 mg/L, 1/34 mg/L, 0·083 mg/L, 24·00 mg/L, 0·083 mg/L and 0·0065 mg/L, respectively. During acclimatation and exposure periods, fish were fed once a day with chilled crustaccan diet (containing cyclops and daphnia) to avoid the starvation effects (Alekseev and Uspendskaya 1974). Solutions were renewed after each 24 hr, to avoid the fouling by food and excretory matter. Controls were also set side by side for comparison.

Fish were sacrificed after 15 and 30 days, and brain, liver, kidney and gills were taken out and pooled separately in ice cold petridishes containing 0·25 M sucrose solution. Tissue homogenates (5%) were prepared separately using 0·25 M sucrose solution, with a Potter Elvehjem homogenizer. Homogenates were centrifuged at 900g under cold conditions (5·0 ± 1·0° C) and supernatants were used for enzyme study. Acid phosphatase activity was measured by the method of Shinowara *et al* (1942). The inorganic phosphate liberated was determined by Fiske and Subbarow (1925) method. Statistical significance of the difference between the control and experimental values was calculated by student's '*t*' test (Fisher 1950).

3. Results and discussion

Average values along with mean ± S.E. of three observations for acid phosphatase activity in brain, liver, kidney and gills of control fishes, and per cent inhibition/stimulation in exposed fishes after 15 and 30 days are given in table 1. It is clear from the table that when fishes were exposed to P, DNP and PCP separately, greater inhibition was observed in fishes exposed to PCP and DNP as compared to fishes exposed to P. This is due to the replacement of hydrogen by chloro and nitro groups in PCP and DNP, respectively (Kopperman *et al* 1974). In general maximum (89·32%) and highly significant ($P < 0·001$) inhibition as observed in brain, and minimum and insignificant (6·93%) in kidney at 1/10 th concentration of (P + DNP)/PCP, respectively after 30 days. However, in kidney biphasic effects of P, and DNP were observed, i.e., inhibition in enzyme activity at higher concentrations and stimulation at lower concentrations. Stimulation was significant ($P < 0·05$) at 1/15th of P, 1/15th and 1/20th of DNP, and at $P < 0·01$ in 1/20th of P after 15 days, and 1/15th and 1/20th of P and DNP after 30 days while it was insignificant at 1/20th of (PCP + DNP)/P combination after 15 days. Inhibition in acid phosphatase in these tissues was in the order,

Table 1. Per cent alterations in acid phosphatase activity in certain tissues of *Notopterus notopterus* exposed to phenolic compounds after 15 and 30 days.

Tissues	Control enzyme activity*	Days	% Alterations in enzyme activity								
			P			DNP			PCP		
			1/10th	1/15th	1/20th	1/10th	1/15th	1/20th	1/10th	1/15th	1/20th
Brain	1·58±0·19		−40·28^b	−31·25^a	−25·54^a	−51·4^c	−39·0^b	−30·3^b	−61·0^c	−45·9^b	−40·6^b
Liver	68·18±2·95		−39·12^b	−30·27^a	−25·53^a	−50·1^c	−39·2^b	−32·6^b	−57·7^c	−48·2^b	−41·0^b
Kidney	3·95±0·41	15	−10·97	+20·91^a	+33·54^b	−28·6^a	+17·3^a	+25·6^a	−49·0^b	−40·8^b	−32·3^a
Gills	2·28±0·21		−40·59^b	−33·28^a	−28·56^a	−53·3^c	−40·6^b	−31·3^b	−60·3^b	−47·2^b	−41·0^b
Brain	1·58±0·26		−53·17^c	−39·28^b	−33·89^b	−60·3^c	−49·5^b	−40·8^b	−79·0^c	−60·8^c	−52·6^c
Liver	68·18±2·95	30	−50·28^b	−42·16^b	−33·59^c	−62·9^c	−48·2^b	−40·6^b	−72·7^c	−60·5^c	−51·2^c
Kidney	3·95±0·41		− 6·93	+28·21^b	+34·56^b	−30·3^b	+26·0^b	+34·3^b	−62·1^c	−49·1^b	−40·6^b
Gills	2·28±0·25		−50·94^c	−43·26^c	−40·54^b	−60·1^c	−48·3^b	−37·5^b	−71·1^c	−58·2^c	−52·2^c
			(PCP+DNP)/P			(DNP+P)/PCP			(P+DNP)/PCP		
Brain	1·58±0·25		−38·2^b	−27·5^a	−24·1^a	−64·5^b	−48·1^b	−40·8^a	−65·6^c	−46·9^b	−39·9^a
Liver	68·15±2·95		−43·1^a	−34·6^a	−30·2^a	−58·8^c	−43·1^b	−34·2^b	−59·3^b	−45·2^b	−38·2^b
Kidney	3·91±0·48	15	−15·3	− 9·8	+10·5	−45·8^b	−38·0^a	−30·9^a	−47·9^b	−33·3^a	−26·4^a
Gills	2·26±0·41		−33·2	−26·8	−22·5	−59·3^b	−45·9^b	−40·1^a	−53·9^b	−40·1^b	−31·7^c
Brain	1·56±0·13		−47·5^c	−36·8^b	−30·1^b	−81·2^c	−63·7^b	−59·5^b	−89·3^c	−68·7^c	−52·1^b
Liver	68·14±2·95		−52·2^c	−33·1^b	−37·2^b	−75·2^c	−58·2^c	−49·2^c	−80·2^c	−60·1^c	−47·7^c
Kidney	3·91±0·40	30	−21·5^a	−13·3	− 8·9	−60·7^b	−48·3^a	−41·5^a	−62·0^b	−40·7^a	−35·5^a
Gills	2·25±0·16		−44·2^a	−35·7^a	−30·2	−74·6^c	−59·0^b	−50·0^b	−70·2^c	−50·2^b	−42·5^b

* Enzyme activity is expressed as μg inorganic phosphate liberated/mg tissue/hr.
Values are mean ± S.E of three observations.
+ indicate per cent stimulation ; − indicate per cent inhibition.
Per cent alteration is statistically significant at a : $P < 0·05$; b : $P < 0·01$; c : $P < 0·001$.

brain > liver > gills > kidney except after 30 days in DNP and after 15 and 30 days in (PCP + DNP)/P where inhibition was in the order, liver > brain > gills < kidney, and after 30 days in P where sequence of inhibition was in the order, brain > gills > liver > kidney.

Phenols enter in blood circulation of fish through gills and skin, and get distributed into different tissues where they affect normal metabolism (Mitrovic *et al* 1968). Dalela *et al* (1980) also studied the effect of sublethal concentrations of P and PCP on hepatic acid and alkaline phosphatases and observed significant inhibition. Synergistic effects of P and DNP on acid and alkaline phosphatases were also observed by Verma *et al* (1980). Authors in this investigation observed that these compounds in combinations showed no definite pattern of toxicity (i.e., inhibition/stimulation in enzyme activity) as they showed separately. At 1/20th, in brain after 15 and 30 days, at 1/10th, 1/15th and 1/20th in liver after 15 days, at 1/15th and 1/20th in liver after 30 days, and at 1/10th in kidney after 15 days, the per cent inhibition was not significantly different in fish exposed to PCP, (DNP + P)/PCP and (P + DNP)/PCP combinations. At 1/15th and 1/20th in kidney after 15 and 30 days, at 1/10th, 1/15th and 1/20th in gills after 15 days, and at 1/15th and 1/20th in gills after 30 days inhibition in fish exposed to (P + DNP)/PCP was significantly lesser than in fish exposed to PCP alone and to (DNP + P)/PCP combination. In fish exposed to (PCP + DNP)/P, inhibition in brain and gills was significantly less as compared to the fish exposed to phenol, in liver inhibition was not significantly different and in kidney significant stimulation ($P < 0{\cdot}05$; $P < 0{\cdot}01$) was there at 1/15th and 1/20th of P both after 15 and 30 days while in (PCP + DNP)/P insignificant stimulation was observed at 1/20th only after 15 days.

Loomis and Lipmann (1948) and Simon (1953) after DNP exposure, and Yap *et al* (1975) and Desaiah (1978) after PCP exposure, pointed out that uncoupling of oxidative phosphorylation is the main cause for inhibition of phosphatases. Uncoupling of oxidative phosphorylation was also pointed out by Dalela *et al* (1980) and Verma *et al* (1980) for the inhibition of acid and alkaline phosphatases. Simon (1953) stated that concentrations higher than those needed to prevent oxidative phosphorylation injured the mitochondrial system so greatly as to block the action of enzymes concerned with oxidative metabolism. Action of uncouplers of oxidative phosphorylation has been pointed out on the basis of chemical (Pressman 1963) and chemi-osmotic (Mitchell 1961) interactions. According to (Pressman 1963), uncouplers promote the conductivity of protons within mitochondrial membranes and subsequently prevent the formation of a gradient across the membrane. According to Mitchell (1961), uncouplers promote the splitting of an energy rich intermediate compound prior to ATP production. Weinbach and Garbus (1969) suggested that uncouplers traverse through lipoprotein layer of mitochondrial membrane and interact with protein groups that then undergo structural changes. It is generally assumed that major changes in mitochondrial function are reflected in morphological alterations and that normal mitochondrial profiles are dependent on the continuing supply of energy rich intermediates produced by oxidative phosphorylation. Weinbach and Garbus (1969) indicated that these uncouplers bind tightly with mitochondrial proteins which are involved in amino acid metabolism. However, authors of this investigation, assumed that all these interactions and processes held simultaneously when

fish were exposed to these chemicals and their combinations, causing the uncoupling of phosphorylation and finally affect the activity of phosphatases. Mixing of chemicals enhances toxicity (synergism) in some cases and decreases (antagonism) in other cases but the actual mechanism of combination effects on acid phosphatase activity is not well-known.

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