Effect of Endrin on the Head Kidney Peroxidase Activity of A Fish, *Anabas Testudineus*

Abstract

Present study was undertaken on inhibition of an enzyme peroxidase activity in the head kidney of a fish *Anabas testudineus*. The effect of cytochrome C on in hited peroxidase activity was also observed. LC_{50} of the endrin was also determined before undertaking the inhibitory effect of the pesticide.

Keywords: Enzyme**,** Peroxidase, Fish, Endrin, Head, Kidney.

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Introduction

Endrin is a chlorinated hydrocarbon and is widely used in controlling pests of paddy, sugarcane, wheat etc. in various regions of India. This pesticide has posed a threat of conta-mination to the fish habitat as run off from the pesticide treated agricultural lands causes pollution of the fishery reservoirs. Peroxidase enzyme plays an important role in thyroid hormone biosynthesis. It oxidises inorganic iodide to active iodine which is then incorporated into the tyrosine moiety—a preliminary step for the synthesis of thyroid hormone. In teleost fish thyroid follicles are not assembled together to form a single gland and large concentration of these follicles are present in the head kidney region has been demonstrated by Chavin (1956), Baker (1958), Baker-Cohen (1959). Guru-mani (1972), Deshpande & Nadkarni (1973). Furthermore Bhattacharya *et. al.*, (1973) and Kumar *et. al*, (1973) recently demon-strated that soluble supernatant fraction (105,000 x g supernatant) from the head kidney of teleost fish possesses very strong peroxidase activity. According to them this peroxidase from the head kidney is physiologically important as it oxidises iodide into triiodide (I_3), which they measure by following the absorbance at 353 nm.

That pesticide and industrial pollutants significantly inhibited the fish brain cholinesterase activity (101 and α-amylase activity (Bhattacharya *et al., 1974*) has also been reported very recently from this laboratory. Hence it seems relevant to observe the effect of endrin, a widely used pesticide, on the head kidney peroxidase activity of a teleost, A, *testuditzeus*. Hzards of pollution noticed Brown *et al. (1969); Karatas et al. (2016); Moraesde et al. (2013); Tamizhazhagam et al.* (2017); Maksymiv *et al., (*2015). J. O. Babayemi, (2016) "Overviewed of Levels of Organochlorine Pesticides in Surface water and food items in Nigeria. Unyimadu *et al.* (2018) assessed the levels of OCPs in fish from Niger river. Levels in the fish may indicate safety for human consumption.

Materials and Methods

A. testudinens was regularly purchased from the local market and adult healthy specimens were only selected for study. The fishes were Initially acclimated in the laboratory aquarium at room temperature for about a week and only those fishes which showed normal activities were selected for the study.

For determination of lethal concentrations and toxicity of the endrin, fishes were exposed in each aquarium in the batches of ten specimens. The test concentration of the endrin were selected from a desilog series and are expressed here in terms of parts of active ingredient per million parts of water (ppm or mg/liter). Testing was conducted in triplicate and each test had a control in which an equivalent amount of water was provided. No fishes died under control conditions, each of the treated and control aqua-rium contained 20 liters of water. Normal level of dissolved oxygen (DO) was maintained by passing air bubbles mechanically inside the aquarium so that effect observed due to endrin

pollution could not be mistaken as due to oxygen depletion. The fildies were sufficiently fed so as to eliminate the possible effect of starvation.

Control and endrin treated fishes were sacrificed and head kidney was dissected out and homogenised separately in Potter-Elvehjem homogenizer with 0.05 M sodium phosphate buffer, pH 5.5. Homogenate thus prepared was subjected to differential ultracentrifugation and l05,00() x g supernatant fraction was collected as enzyme. Detail of the fractionation procedure have already been reported elsewhere (Kumar *et al.* 1973).

The peroxidase activity of the head kidney was measured by following the increase in optical density with guaiacol as the hydrogen donor. The reaction mixture contained 150 µ moles of sodium phosphate buffer pH 5.5, 1 µ, mole of guaiacol, H_2O_2 1.2 µ. mole and suitable volume of enzyme preparation and water to make a final volume of 3.0 ml. The enzyme was added last to start the reaction and the optical density was measured against an enzyme blank for 60 sec. at 10 sec. intervals. Enzyme protein was measured according to the method of Lowry et. al. (1951) by using bovine serum albumin as the standard.

Results given in tables awl figures represent single experiment, but each observation was verified by at least three experiments.

Results and Discussion

In order to determine the lethality of endrin experiments were carried out in different aquarium with varied concentration of endrin and maintain-ing a control in each case. Two time-sequence sets of experiments were under investigations one for 48 hours and another for 72 hrs to. determine the LC_{50}„ concentration of endrin. Table 1 shows 48 hrs Lei3O* level of endrin was 0.00025 ppm and 72 hours LC_{50} 0 level was 0.00015 ppm. In comparison to the other toxic pollu-tants the degree of concentration which caused the mortality of the fishes by endrin is 'considerably low. This obviously suggest the high potentiality of endrin in causing the damage to the fishes.

Different parameters for the present enzyme such as pH optima, optimum hydrogen ion concentration and about its location in soluble supernatant fraction has been reported earlier (Kumar et. al, (1973)). Control and endrin treated *.A. testudineus* (48 hrs & 72 hrs LC_{50} survived fishes) were sacrificed and head kidney was dissected out homogenised and differentially centrifuged separately to obtain supernatant fraction. Control and endrin treated head kidney soluble enzyme was then assayed separately for the determination of peroxidase activity. The result of this study was recorded in Table 2. In both the 48 hrs and 72 hrs LC_{50} . Endrin treated fishes peroxidase activity of the head kidney soluble supernatant were significantly inhibited. This inhibition was more marked in the case of 48 hrs LC_{50} fishes. Attempt has been made to demonstrate whether the toxic effect of endrin could be counteracted by other chemi-cal agents or not. Cytochrome C is a known stimulator of peroxidase enzyme and it has boon demonstrated earlier that fish head kidney peroxidase activity was greatly stimulated by the addition of cytochrome C (Furthermore Bhattacharya *et. al*. 1973). It was, therefore, thought that addition of cytochrome C might minimise the inhibitory effect of endrin. To observe the effect of cytochrome C on the endrin inhibited peroxidase, endrin treated fishes (48 hrs LC_{50}) were removed in aquaria containing varied concentra-tions of cytochrome C and exposed for 24 hours. Table 3 shows that when cytochrome C was administered in increasing concentrations regaining of the lost enzyme activity occurred. At the concentration of 0.001 ppm cytochrome C completely abolishes the inhibitory effect of endrin. The mechanism, as to how cytochrome C removed the inhibitory effect of endrin is not known and further work is needed to explain the phenomenon completely.

Conclusion

Present head kidney peroxidase is physiologically important which oxidises inorganic iodide into an active iodine (Kumar et. al, (1973)) the inhibition therefore suggest that endrin will cause hindrance in the formation of active iodine which again will result in the depletion of thyroid hormone synthesis. Again the inhibition of peroxidase activity by endrin was found to be noncompetitive one as its inhibitory action could not be reversed by increasing the concentration of substrate. An explanation regarding the mechanism of inhibition can be made front this study that endrin probably blocks the active site of head kidney peroxidase and thus retarding enzyme-substrate reaction. However, it is too premature to suggest any conclusion in this regard.

Table- 1

Determination of LC_{50} endrin concentration

No. of test animal	LC_{50} determined in hrs	Concentration of endrin (ppm)
Ten	48	0.00025
Ten	72	0.00015

Table- 2

Effect of endrin on head Kidney soluble supernatant peroxidase

System	Δ OD/min/mg protein	% of inhibition
Control	4.5	–
Treated (48 hrs Lc_{50})	0.8	82.2
Treated (72 hrs Lc_{50})	1.2	73.3

Table- 3
Effect of cytochrome c on 48 hrs. LC_{50} endrin treated fish peroxidase activity

Concentration of cytochrome C in ppm	∆ OD/min/mg protein
0.00001	0.85
0.0001	2.35
0.001	4.45

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