EFFECT OF MERCURIC CHLORIDE ON THE ACTIVITY OF ALKALINE PHOSPHATASE ENZYME IN OVARY OF FISH, *HETEROPNEUSTES FOSSILIS* AND RECOVERY BY CHLORELLA

NEHA PANDIT, LATA BHATTACHARYA

Abstract: The effect of mercuric chloride on alkaline phosphatase (ALP) enzyme activity in ovary of fish *Heteropnuestes fossilis* was analyzed histochemically. Heavy metal contaminants in aquatic ecosystems pose a serious environmental hazard because of their persistence and toxicity. Among the heavy metal pollutants, mercury receives a special attention. The greatest use of mercury include in the production of electric apparatus; in chlor-alkali industries; in pharmaceuticals; in the pulp and paper industries as a slimicide and in the agricultural fungicides. In the present study the fish ovary of control group exhibited positive reaction to alkaline phosphatase enzyme. *Heteropnuestes fossilis,* when treated with 0.01 ppm of $HgCl_2$ for 21 days exhibited decreased activity of alkaline phosphatase enzyme, indicated by negative reaction in ovarian follicles. After giving Chlorella with food to mercuric chloride pretreated fishes, moderate to intense reaction was observed in ovarian follicles. Thus, the Chlorella was effective to recover this enzyme activity.

Keywords: Alkaline phosphatase, Heteropnuestes fossilis, Mercuric chloride, Ovary, Chlorella.

Introduction: Heavy metal contaminants in aquatic ecosystems pose a serious environmental hazard because of their persistence and toxicity. Among the heavy metal pollutants, mercury receives a special attention. The greatest use of mercury include in the production of electric apparatus; in chlor-alkali industries; in pharmaceuticals; in the pulp and paper industries as a slimicide; in the agricultural fungicides and in the production of plastic [5].

Enzymes are the proteins which are soluble colloidal organic catalysts formed by living cells. Enzymes play an important role in the growth, digestion, respiration, spawning and other processes in fish. The alkaline phosphatase (ALP) is a group of functionally related enzyme, hydrolyze organic phosphates in the pH range of 9.0 to 10.5 [26]. These enzymes are involved in a variety of metabolic activities such as permeability, growth and cell differentiation, protein synthesis, absorption and transport of nutrients, gonadal maturation, and steroidogenesis [17].

Herbal antioxidants are quite useful in heavy metal detoxification. Chlorella is a micro-alga. It contains 50-60% amino acid, vitamins, beta carotene etc. Chlorella was used in aquaculture as a feed for fish. A little information is available on their use to detoxify mercury from fish body. The aim of the present study is to explore the alterations in alkaline phosphatase enzyme activity histochemically in female gonad, ovary of *H. fossilis*, after exposing the fish with mercuric chloride and also the recovery responses after giving chlorella with diet of fish for 21 days duration.

Material and methods: Experimental Animal: *Heteropnuestes fossilis* (Bloch).

Toxic agent: Mercuric chloride $HgCl_2$, Mol. Weight: 271.50

Recovery agent: Chlorella (Powder form)

Methodology: Living, healthy, mature female fish, *H. fossilis* were procured from local fish market of Ujjain, (M.P.) and used as the test specimen. The fish were acclimated to standard laboratory conditions for a period of 10 days prior to the experiment. The fish were treated with 0.01% of $KMno_4$ solution to remove any dermal infection. The average weight and length of fish were 25±5 gm and 12±5 cm respectively.

Fishes were divided into following three groups:

S.No.	Group	Treatment
1	Control	Water without $HgCl_2$ + plain food
2	Treated	Exposed to 0.01ppm $HgCl_2$ + plain food
3	Recovery	Exposed to 0.01ppm $HgCl_2$ + chlorella (5% of diet) with food

Fishes of all experimental groups were fed on dried and chopped prawn, twice a day. The daily dose of food for fish was 30 mg/fish/day. Aquarium water was changed on every third day of all aquaria. After changing the water, $HgCl_2$ was added in treated and Recovery group of aquarium water. Water was aerated by an aquarium pump for 30 minutes daily. The daily dose of recovery agents for fish was 5% of their food. Fishes of all experimental groups were sacrificed after 21 days. Ovaries were separated and

processed for further studies.

Histochemical localization of alkaline phosphatase activity was observed in ovary of all experimental groups, by using Gomori's alkaline phosphatase - Cobalt Method [7]. Sites of alkaline phosphatase activity are black. All the results for final observation were processed in the form of microphotographs. The microphotographs of sections were taken using Leica ATC 2000 light microscope with MINTRON auto iris capture unit and PCTV Vision software.

Results and Discussion: In the present study the alkaline phosphatase enzyme activity was observed in ovary histochemically. Alkaline phosphatase is an intrinsic plasma membrane enzyme found in almost all animal cells [11].

Enzymes are biochemical molecules that control metabolic processes of organisms [20]. Change in the activity of enzymes by heavy metals and pesticidal stress lead to metabolic disturbances [6],[8]. Thus, by estimating enzyme activities in an organism one can easily identify disturbances in its metabolism. Enzymes are relatively fragile substances with a tendency to undergo denaturation and inactivation under adverse conditions [9]. Phosphatase is known to be sensitive to metal exposure and can be used to predict metal toxicity. The knowledge of effects of heavy metals on enzymatic activities in fish is very important to describe the health of fish status and to understand the ecological impacts [16].

It is important to study the activities of acid phosphatase and alkaline phosphatase enzymes because they are metalloenzymes. They involved in various metabolic processes such as permeability, growth and cell differentiation, protein synthesis, absorption of nutrients and gonadal maturation [17]. Any change in the activities of both the enzymes can affect these processes. These enzymes have been used as bioindicators of heavy metal intoxication because of their sensitivity to metal pollution [2],[13].

In the present study, the responses of alkaline phosphatase enzyme activity to mercuric chloride exposure in ovary of catfish, *H. fossilis* was investigated. Recovery process of this enzyme was evaluated in same period by using chlorella. The recovery of enzyme activity was not mentioned till the day, therefore, the phosphatase recovery phase of the present experiment will extend our knowledge of the reversibility of enzyme activity in fish. In toxicological studies acid phosphatase and alkaline phosphatase are important biochemical enzymes to be used to detect the alterations of physiological metabolism of animals induced by metal exposures [19],[15].

In 21 days control group the oocytes of vitellogenic phase exhibited intense reaction. Their follicular surrounding layer and cytoplasm showed positive reaction to alkaline phosphatase enzyme. The yolk material of mature oocytes showed strong positive reaction (Fig.1). On the other hand, after the exposure of mercuric chloride the fish ovary exhibited reduced activity of alkaline phosphatase enzyme. The degenerated hypertrophied granulosa layer showed negative reaction in almost all the mature oocytes. The cytoplasmic contents of different primary and growing oocytes showed very weak localization of alkaline phosphatase enzyme (Fig.2). The present study shows the inhibition of alkaline phosphates activity in gonadal tissues of *H. fossilis* exposed to mercuric chloride. These results supported by the findings of [24]. Reference [21] noted that mercuric chloride treatment reduced the ovarian alkaline phosphatase in *Clarias batrachus*.

An attempt to find a role between enzymatic activity and gonadal development in female fish, *H. fossilis*, the present study revealed that in control fish the alkaline phosphatase activity in the granulosa cells suggests that the cells of this layer are involved in the transported of yolk proteins to the developing oocytes. Our findings are in good agreement with the findings of [1], according to them the alkaline phosphatase is present on all cell membranes where an active transport occurs. Decrease in alkaline phosphatase enzyme is possibly due to leakage of the enzyme from cytosol across the damaged plasma membrane into general blood circulation or decreased enzyme synthesis on account of organ dysfunction. Intoxication with cadmium caused significant reduction in alkaline phosphatase enzyme in liver and kidney of catfish, *H. fossilis*, possibly due to damage and dysfunction of these organs [22]. Decrease in alkaline phosphatase activity may be taken as an index of tissue damage and necrosis [14]. The alkaline phosphatase enzyme is a ubiquitous plasma membrane bound enzyme, is often employed to assess the integrity of the plasma membrane [1] and any perturbation in the membrane property caused by interaction with xenobiotics could lead to alteration in alkaline phosphatase activity [12]. Decreased alkaline phosphatase activity might be attributed to the loss of alkaline phosphatase from plasma membrane into extra cellular fluid [10] and the reduction in concentration or total absence of specific phospholipids required by this membrane-bound enzyme to express its full activity [27], under the interaction of heavy metal with plasma membrane or due to inhibition of the enzyme activity at the cellular/molecular level [1]. Reference [22] found a decreased activity of alkaline phosphatase enzyme in ovary of *Channa punctatus* exposed to mercuric chloride. Our result corroborate with these findings. The possible mechanism of inhibition of the enzyme may involve either the effect of toxins or its

metabolites on enzymes themselves or competition with the substrate or the reversal of the enzyme by affecting the factors like Mg^{++} etc. Reference [18] reported that the binding of toxins with enzymatic proteins, leaving the apoenzyme, could be the possible cause of decrease in phosphatase activity. The results of present study agree with the above findings. However the tendency of fishes to augment and resume back normal levels of alkaline phosphatase in gonads reflects the presence of compensatory mechanisms in a changing environment.

In chlorella recovery group, the activity of alkaline phosphatase enzyme was greatly improved. Some of the oocytes gave very strong reaction in their surrounding layer and ooplasm but negative reaction was not seen that were occupy in treated group (Fig.3). Few of them showed moderate reaction. Alkaline phosphatase is zinc and magnesium dependent enzyme [4] and both these metals (Zn and Mg) are significantly removed from tissues by mercury [3], therefore recovery of such a mercury sensitive enzyme is a good evidence to judge the therapeutic effect of any antidote. The present study showed recovery of alkaline phosphatase enzyme in gonads of fish of chlorella recovery group. However time duration is important to be the best in restoration the normal condition of animals. The gonads (testis and ovary) showed increasing activity of alkaline phosphatase when mercuric chloride treated fish fed on algae chlorella.

The mercuric chloride treatment affects negatively the alkaline phosphatase enzyme activity in ovarian tissue. The ALP enzyme activity was inhibited due to mercury. But chlorella helps to increase this enzyme activity. It was effective to restore the enzyme activity in mercuric chloride pretreated fishes. The fluctuations of the enzyme in animal tissues during mercury intoxication create physiological and pathological alterations [25]. Therefore it is necessary to maintain its normal level in all the tissues of animals. Thus, the present study attempted to restore the enzyme activity in female gonad of *H. fossilis* when fed on algae, chlorella. These recovery responses might be due to the effective role of the contents that present in chlorella.

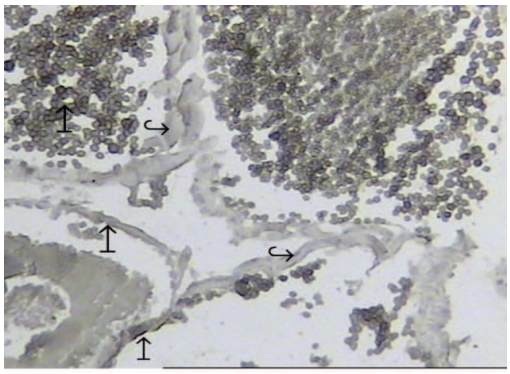

Fig.1

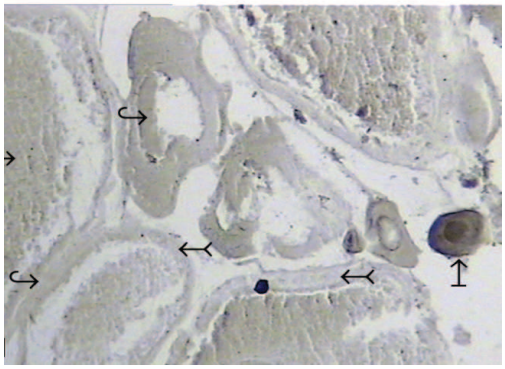

Fig.2

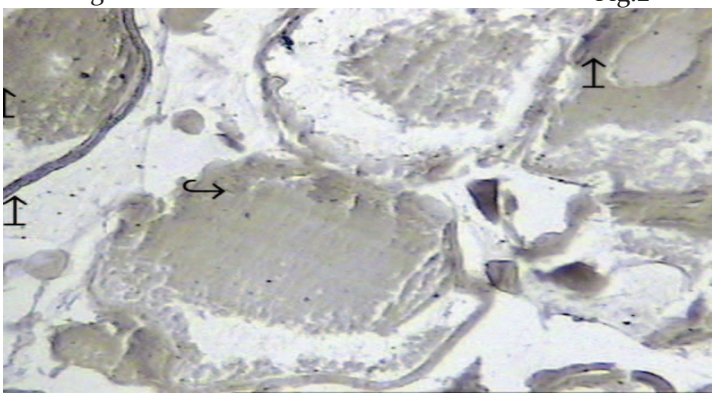

Fig.3

Microphotographs of transverse section of ovary of female fish, *H. fossilis* showing localization of alkaline phosphatase enzyme (ACP) activity (21 days, X 400)

Fig.1 Control group: Showing an intense reaction in surrounding layer and in yolk material of mature oocytes
Fig.1 Treated group: Showing decreasing trend of ALP enzyme activity in different growing oocytes
Fig.3 Chlorella recovery group: Showing intense reaction in outer covering of oocytes and moderate reaction in ooplasm

\- Negative reaction

\- Intense reaction

\- Moderate reaction

References:

1. Akanji, M.A., Olagoke, O.A. and Oloyede, O.B. (1993): Effect of chronic consumption of metabisulphite on the integrity of rat kidney cellular system. *Toxicol*., 8: 173-179.
2. Anan, Y., Kunito, T., Ikemoto, T., Kubota, R., Wanatabe, I., Tanabe, S., Miyazaki, N., Petrov, E.A. and Petrov, E.A. (2002): Elevated concentrations of trace elements in Caspian Seals (*Phoca caspica*) found stranded during the mass mortality events in 2000. *Arch. Environ. Contam. Toxicol.*, 42: 354-362.
3. Bapu, C., Purohit, R.C. and Sood, P.P. (1994): Fluctuations of trace elements during MMC toxication and chelation therapy. *Human Exp. Toxicol.*, 13: 815-823.
4. Ciancaglini, P., Pizauro, J.M., Grecchi, M.J., Curti, C. and Leone, F.A. (1989): Effect of Zn (II) and Mg (II) on phosphohydrolytic activity of rat matrix-induced alkaline phosphatase. *Cell Mol. Biol.*, 35 (5): 503-510.
5. Clarkson, T.W. and Marsh, D.O. (1982): Mercury toxicity in man. In A.S. Prasad (ed.) *Clinical, biochemical and nutritional aspects of trace elements*. Alan R. Liss. Inc., New York. 6: 549-568.
6. Fatima, M., Mandiki, S.N., Douxfils, J., Silvestre, F., Coppe, P. and Kestemont, P. (2006): Combined effects of herbicides on biomarkers reflecting immune-endocrine interactions in goldfish, immune and antioxidant effects. *Aquat. Toxicol.*, 77: 11-19.
7. Gomori, G. (1939): Microtechnical demonstration of alkaline phosphatase in tissue sections. *Proc. Soc. Exp. Biol. Med.*, 42: 23-25.
8. Lissandra, G., Denise, S.M., Marcia, C., Milene, B.F., Fabio, A.P., Marta, F.D. and Vania, L.P. (2006): Effect of glyphosate herbicide on acetylcholinesterase activity and metabolic haematological parameters in piava, *Leporinus obtusidens. Ecotoxicol. Environ. Saf.*, 65: 237-241.
9. Lopez, L.E., Favari, L., Martinez, T.L., Madrigal, M. and Soto, C. (2003): Hazard assessment of a mixture of pollutants from a sugar industry to three fish species of western Maxico by the responses of enzymes and lipid peroxidation. *Bull. Environ. Contam. Toxicol.*, 70: 739-745.
10. Malbica, J.O. and Hart, L.G. (1971): Effect of adenosine triphosphate (ATP) and some anti-inflammatory agents on purified lysosomal fraction having high acid phosphatase and labile glucuronidase activity. *Biochem. Pharmacol.*, 20: 2017-2026.
11. Mazorra, M.T., Rubio, J.A. and Blasco, J. (2002): Acid and Alkaline phosphatase activities in the clam *Scrobicularia plana*: kinetic characteristics and effects of heavy metals. *Comp. Biochem. Physiol. B*, 131: 241-249.
12. Molina, R., Moreno, I., Pichardo, S., Jose, A. Moyano, R., Monterde, J.G. and Camean, A. (2005): Acid and alkaline phosphatase activities and pathological changes induced in tilapia fish (*Oreochromis sp.*) exposed subchronically to mycrocystins from toxic cyanobacterial blooms under laboratory conditions. *Toxicol*., 46: 725-735.
13. Mora, S.D., Sheikholeslami, M.R., Wyes, E., Azemard, S. and Cassi, R. (2004): An assessment of metal contamination in coastal sediments of the Caspian Sea. *Mar. Pollut. Bull.*, 48: 61-77.
14. Onikienko, F.A. (1963): Enzymatic changes from early stages of intoxication with small doses of chloroorganic insecticides. Gigienari. *Fiziol. Truda. Toksikol. Klinikackiaev Gos. Iz. Med. Git. Ukr. USSR*, 77.
15. Oruc, E.O. and Uner, N. (1999): Effects of 2,4 diamin on some parameters of protein and carbohydrate metabolism in serum, muscle and liver of *Cyprinus carpio. Environ. Pollt.*, 105: 267-272.
16. Radhaiah, V., Girija, M., Rao, K.J. (1987): Changes in selected biochemical parameters in the kidney and blood of the fish, *Tilapia mossambicus* (Peters), exposed to heptachlor. *Bull. Environ. Contam.Toxicol.*, 39: 1006-1011.
17. Ram, R.N. and Sathyanesan, A.G. (1985): Mercuric chloride, Cythion and ammonium sulphate

induced changes in the brain, liver and ovarian alkaline phosphatase content in the fish *Channa punctatus*. *Environ. Ecol.* 3: 263-268.
18. Rana, S.V.S. and Kumar, A. (1980): Enzymological studies on the liver of rats fed with molybdenum and copper. *Toxicol. Lett.*, 6: 163-166.
19. Reddy, P.S. and Bhagyalakshmi, A. (1994): Changes in oxidative metabolism in selected tissues of the crab (*Scylla serrata*) in response to cadmium toxicity. *Ecotoxicol. Environ. Saf.*, 29: 255-264.
20. Roy, S.S. (2002): Some toxicological aspects of chlorpyrifos to the intertidal fish, Boleopthalmus dussumieri. Ph.D. Thesis University of Mumbai, India.
21. Saksena, D.N. and Agrawal, A. (1991): Effect of mercuric chloride on certain biochemical constituents of the ovary of Indian fresh water catfish, *Clarias batrachus*. *J. Adv. Zool.*, 12: 12-18.
22. Sastry, K.V. and Agrawal, M.K. (1979): Mercuric chloride induced enzymological changes in kidney and ovary of a teleost fish, *Channa punctatus*. *Bull. Environ.Contam. Toxicol.* 22: 38-43.
23. Sastry, K.V. and Subhadra, K. (1985): *In vivo* effects of cadmium on some enzyme activities in tissues of the freshwater catfish, *Heteropneustes fossilis*. *Environ. Res.*, 36(1): 32-35.
24. Sood, P.P. and Unnikumar, K.R. (1987): Effect of N-acetyl DL-homocystein thiolactone and dimercaptosuccinic acid on the restoration of ACP in the nervous system of rat during methyl mercury toxication. *J. Environ. Pathol. Toxicol. Oncol.*, 7: 21-28.
25. Vachharajani, K.P., Unnikumar, K.R. and Sood, P.P. (1986): Effect of methylmercuric chloride on the acid phosphatase activity in liver and kidney of rat. *Ad.Bio.Sci*. 5: 155-160.
26. Vimla, G. and Anbu, K. (2011): Comparative study on the enzyme profile of monocrotrophs treated Indian major carp. *Proc. Nat. Acad. Sci. India*. Section B 81(IV): 389-395.
27. Yukubu, M. T., Olatunji, I.K. and Akanji, M.A. (2002): Comparative effects of administration of halofantrine hydrochloride and dihydroartemisin in on some enzymes of selected rat tissues. *NISEB J.*, 2: 175-180.

***

Neha Pandit/Lata Bhattacharya /
Govt. Guest faculty/ Zoology Department/ S.B.N. Govt. P. G. College/ Barwani/
/Professor/ School of Studies in Zoology and Biotechnology/Vikram University/
Ujjain/ panditnehazoo22@gmail.com