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Effect of cisplatin on acid phosphatase activity of different tissues of freshwater bivalve, *Corbicula striatella (D).* 1854)

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Abstract
Cisplatin are anticancer drug which action with potent and effective for chemotherapy against solid tumors. These drugs show effective chemoprevention in chemotherapy and also lead to several manipulations and cytotoxicity in tissues. In present studies, sub-lethal doses of Cisplatin (LC50/10 for 96 hours) were given to freshwater bivalves, *Corbicula Striatella*. For 45 days. The acid phosphatase activity was determined from various tissues of control and experimental bivalves by method of Gutman and Gutman. It was found that acid phosphatase activities were increased in various tissues with increased period of exposure to anticancer drugs in experimental bivalves. It was also observed that acid phosphatase activity increased in various tissues in Cisplatin treated bivalves.

Keywords: anticancer drugs, acid phosphatase, cytotoxicity, bivalves

Introduction
All enzymes are biological catalyst which is regulate chemical reactions. Chemically proteins produced specifically to carry out specific catalytic reaction. Every step in a pathway of biochemical reaction is always catalyzed by a specific enzyme. The absence of any one enzyme can arrest the pathway and proves to be fatal physiological defect. The actual catalytic site of an enzyme molecule is a small part where the amino acid component are arranged precisely to bind its substrate and thus forming enzyme substrate complex to convert it into the particular product. The substance that binds with the enzyme and decreases the rate of enzyme catalyzed reaction is called as an enzyme inhibitor. Enzyme catalyzed reaction depends partly on how the enzyme and substrate will bind together to form enzyme substrate complex. Bivalve molluscs form important aquatic biota, where anticancer drugs can enter into the body of molluscs and interfere with the normal enzyme action which can lead into many physiological and biochemical changes in the body. All freshwater organisms when exposed to toxicants for even a short duration of time leads to considerable destruction of the internal organs with respect to enzymatic components. Enzyme assays and estimation of metabolites have been proposed as a most acceptable biochemical mean for monitoring toxicity of anticancer drugs. A normal regulatory mechanism ever tries to overcome inhibitory action to maintain the overall fitness of the body of an organism. The possible mechanism in cisplatin induced nephrotoxicity has been attributed to reactive oxygen species [1]. Therefore, the freshwater bivalve, *Corbicula striatella* is selected as an experimental model for the enzyme study. The first platinum antitumor agents were found as a result of study of effects of electric current on the bacterial growth, where growth inhibition was found to occur but it was due to platinum complex of ammonia and chloride produced in the culture medium at the platinum electrode. Although this antimetabolite is toxic, its positive effect in chemoprevention makes it one of the most popular anticancer drugs used for treatment against solid tumors. All enzymes are chemically proteins in nature and control various sub cellular functions [2].

Acid phosphatase
Acid phosphatase is a nonspecific monoesterase, regarded as the biological marker enzyme. It has been found in lysosome and Golgi cisternae. Acid phosphatase, a lysosomal enzyme, hydrolyses phosphate esters in acidic medium. It also catalyzes the transfer of phosphoryl groups. Ide and Fischman [3] suggested that the lysosomal enzymes get involved in many metabolic transformations in vivo. The changes in acid phosphatase activity in various organs of snails which serve as an intermediate host for trematode parasites have been reported by number of workers. Acid phosphatase enzymes are responsible for transphosphorylation and play an important role in overall energy metabolism of an organism. Cheng [4].

Impact of Anticancer drugs on Tissue Phosphatase Activity
Influence of anticancer drugs on a series of physiological reactions can enable to establish specific response. High level of toxic chemical compounds brings about the adverse effects on aquatic organisms at molecular or cellular level and leads to imbalance in biochemical components, which become useful in determination of different toxicants and protective mechanisms of the body to combat the toxic effect of the substances. In addition to anticancer drugs, many drugs induce the apoptosis, under such acid phosphatase activity increases. Chronic exposure to anticancer drugs, Cisplatin increased the acid a phosphatase activitiy in various tissues of freshwater bivalve, Corbicula strietella [5]. Hence these enzymes are used as diagnostic enzymes in clinical analysis work. The damaged RNA and DNA are also vulnerable to the RNAse and DNAse attacks respectively.

Methodology

The freshwater bivalves, *Corbicula Striatella* (D) were collected from Girna lake area near Jamda, which is 14 km away from Chalisgaon, District Jalgaon of Maharashtra State. Bivalves were collected and brought to laboratory in aerated container. The bivalves were cleaned and kept in glass aquarium. They were maintained in a glass aquarium containing dechlorinated water for 3- 4 days at 21^0C - 26^0C temperature. The PH of water was in the range of 7.0 - 7.5 and well acclimatized at laboratory conditions. The water in aquarium was changed regularly after every 24 hours. After acclimatization, healthy bivalves with size ranging from 2.8-3.00 cm height X 4.6-5.3 cm length were selected from the aquarium and used for the experiments. The well acclimatized bivalves, *Corbicula Striatella* were divided into three groups with equal number of animals. They were kept in separate aquarium for 45 days. Bivalves from one of the three groups were not exposed to anticancer drugs and were maintained as a control. Out of remaining two groups, one was treated by chronic concentration (LC50/10 value of 96 hours) of Cisplatin, 1.007 ppm. On 15th, 30th and 45th day of exposure, bivalves from each experimental group were dissected. The tissues such as gonads, digestive glands, mantle and foot were removed and kept in ice cold condition. Then 01% homogenate of each tissue was prepared in ice cold buffer. Then 01% homogenate of each tissue was prepared in ice cold buffer. The homogenate was centrifuged, and supernatant removed was used to determine the acid phosphatase activity.

Acid phosphatase activity

Acid phosphatase activity of different tissues was estimated by the method of Gutman and Gutman [18]. The enzyme activity was carried out in reaction mixture containing 01 ml (0.01M) substrate Disodiumphenyl phosphate, 2 ml citrate buffer with PH 4.9- and 0.5-ml ice cold tissue homogenate. The reaction mixture was incubated at 37^0C for one hour. The reaction was terminated by adding 1 ml of Folin Ciocalteu's phenol reagent and reaction mixture was centrifuged at 3000 rpm for 10 minutes. Then 2 ml of 15 % sodium carbonate was added in each test tube of three repeats. The blue color complex developed was read at 660 nm on colorimeter. The blank readings were taken without incubation of reaction mixture. The initial reading of the reaction before incubation was subtracted from the final reading of the enzyme activity after the incubation. The calibration of standard graph was developed by using phenol as a standard. The activity of acid phosphatase enzyme was expressed as KA units/100 gm. of fresh tissue/ hour at 370C at PH 4.9. (K.A. unit = King Armstrong unit). Standard deviation and student 't' test of significance were calculated and expressed in respective tables**.**

Results

of sub lethal concentration of cisplatin (1.007 ppm) on acid phosphatase activity was studied in tissues such as gonads, digestive glands, mantle and foot of freshwater bivalve, *Corbicula Striatella*. Acid phosphatase activity determined is given in the Table. The enzyme activity of acid phosphatase was expressed in KA units / 100-gram fresh tissue / hour at 37^0C. Standard deviations of five repeats were calculated and are presented in the table. Student-'t' test and percentage increase or decreases in the enzyme activities are also given in the table.

Table 1: Acid phosphatase activity in different tissues of *Corbicula striatella* on exposure to chronic dose of Cisplatin.

Sr. No.	Tissues	Exposure to	15 days	30 days	45 days
1)	Gonads	Control	15.70±0.845	16.20±1.945	15.43±2.341
		Cisplatin (0.836 ppm.)	18.50±1.930* (+22.06)	19.20±1.816* (+26.27)	21.43±2.201* (+46.52)
2	Digestive Gland	Control	14.48±1.845	14.20±2.489	13.43±.1.214
		Cisplatin (0.836 ppm.)	15.15±1.987* (+18.45)	18.20±1.947* (+15.65)	20.43±2.487*** (+34.57)
3	Mantle	Control	8.09.23±0.845	8.20±1.945	07.43±2.341
		Cisplatin (0.836 ppm.)	10.21±0.458** (+34.46)	11.78±1.487* (+49.53)	12.74±0.28* (+74.71)
4	Foot	Control	04.43±2.536	04.20±0.456	04.43±2.346
		Cisplatin (0.836 ppm.)	06.94±0.845* (+16.46)	06.20±0.945*** (+33.46)	06.43±4.341* (+64.46)

1. Values are expressed in K.A. units /100 gm of wet tissue/hour at 37 0C. 2. ± indicates S.D. of five observations. 3. (+) indicates % increase over control. 4. Significance of t-test: *P<0.05**P<0.01, ***P<0.001, NS=Non -significant

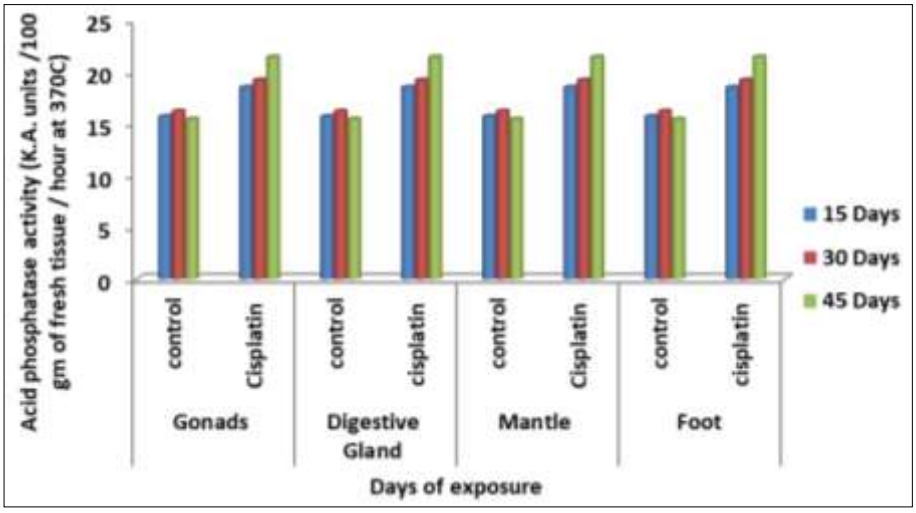

Fig 1: Acid phosphatase activity (K.A. units /100 gm of fresh tissue / hour at 37 ^{0}C) in different tissues of *Corbicula Striatella* after chronic exposure to Cisplatin.

Discussion

Acid phosphates is non-specific monoester. The acid phosphatase enzymes are responsible for transphosphorylation and playing an important role in the general energy metabolism of an organism. The activities of acid phosphatase on chronic exposure to anticancer drugs, Cisplatin was found to be increased in various tissues of *Corbicula striatella* indicates the effect of the drugs on the cells with high metabolic rate. Increase or decrease in the enzyme activities represents the stress condition on an organism that results into burden on body metabolism.

In the present study, it was observed that after chronic exposure to cisplatin (1.007 ppm. the enzyme activities of acid phosphatase was found to be increased significantly ($p<0.05\%$) in mantle, foot, gonads and digestive glands of experimental bivalves, *Corbicula striatella* as compared to those of control group of bivalves. It was also observed that, the increase in enzyme activities was found to be more in gonads and digestive glands than mantle and foot tissues of experimental group of bivalves, probably due to high rate of metabolism. As cisplatin damage the nucleic acid particularly DNA, the cells become morbid and thus to recycle the phosphates, the level of these enzymes increase in the cells. The activity of acid phosphatase in chronic exposure to anticancer drugs cisplatin was found to be increased in various tissues of freshwater bivalve, *Corbicula striatella* indicating the effect of the drugs on the cells with high metabolic rate. Norseth [7] reported decrease in acid phosphatase activity due to bioaccumulation of mercury in the lysosomes, and blockage in the availability of enzyme. Generally, the acid phosphatase activity increases due to induced condition and inhibition of enzyme, which would remain in latent state inside the membrane of lysosomes, due to damage of the membrane [8]. Acid phosphatase is regarded as the marker enzyme; it has been found in lysosomes and Golgi cisternae. Acid phosphatase enhances the rate of metabolism and transphosphorylation [9]. Sensitization of cells in tissues may induce proliferation of smooth endoplasmic reticulum in digestive glands and resulted in elevated production and release of acid phosphatase [10] Increased acid phosphatase activity indicates the increased apoptosis and nucleic acid digestion in the Cisplatin treated bivalves. Increased acid phosphatase activities in various tissues of *Corbicula striatella* indicate the increased apoptosis and nucleic acid digestion in the Cisplatin treated bivalves [11].

Conclusions

1. Cisplatin are used as anticancer drugs for the control of neoplastic growth. The effects of the anticancer drugs on the enzyme activity were studied on the experimental model animal freshwater bivalve, *Corbicula Striatella*. 2. The effect of chronic concentration (LC50/10 value of 96 hours) of Cisplatin (1.007 ppm). In acid phosphatase activity in gonads, digestive glands, mantle and foot of *Corbicula Striatella* was studied. 3. Acid phosphatase activity in gonads, digestive glands, mantle and foot of *Corbicula Striatella* were found to be increased significantly on chronic exposure to Cisplatin.

4. The Cisplatin inhibiting the replication and transcription may induce the apoptosis and hence the activity of enzyme acid phosphatase increases in gonads, digestive glands, mantle and foot of *Corbicula Striatella*. 5. Increase in acid phosphatase enzyme activity was found to be more in gonads and digestive glands than that of mantle and foot of experimental bivalves might correlate to rate of metabolism.

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