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Effect of Cisplatin and 5- Fluorouracil on Acid Phosphatase activity in different tissues of fresh water bivalve, *Parreysia corrugata* (M)

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ABSTRACT

Cisplatin and 5-Fluorouracil are potent and effective anticancer drugs commonly used 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 and 5-fluorouracil (LC_{50}/10 for 96 hours) were given to fresh water bivalves, *Parreysia corrugata* for 30 days. The acid phosphatase activities were determined from different tissues of control and experimental bivalves by method of Gutman and Gutman. It was found that acid phosphatase activities were increased in different tissues with increased period of exposure to anticancer drugs in experimental bivalves. It was also observed that acid phosphatase activity increased in different tissues were found to be more in Cisplatin treated bivalves than that of 5- fluorouracil treated bivalves.

Key Words: Anticancer drugs, acid phosphatase, Cytotoxicity, Bivalves.

INTRODUCTION

Biochemical reactions in all living organisms occur rapidly at optimum temperatures and under moderate conditions of PH, pressure etc., this basically happens because of the metabolic action of biological catalysts called as an enzyme. All enzymes are 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 will the enzyme and substrate bind together to form enzyme substrate complex. The amount of which gives the rate of activityof the enzyme per unit time. An enzyme alkaline phosphatase proposed to study enzyme activity is very important in recycling phosphate in the living cells. This seems to be prevalent particularly in tissues which are engaged in transport of nutrients.
Mollusc bivalves are the aquatic organisms representing submerged benthic fauna of marine and fresh water resources. 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 fresh water 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. Most of the enzymes which are functional in different metabolic pathways have shown altered pattern of enzyme activities due to exposure of anticancer drugs. Certainly, this is the indicator of functional disorders. 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 (ROS) [1]. ROS is a currently recognized mechanism in the pathogenesis of the cisplatin induced testicular toxicity in experimental study [2],[3]. Cisplatin causes lipid peroxidation (LPO) and decreases the activity of enzymes that protects against oxidative damage in testicular tissue from cisplatin treated rats [4]. Oxidative damage caused by ROS has been implicated in the pathogenesis of cisplatin induced testicular injuries [5].

Therefore, the fresh water bivalve, *Parreysia corrugata* is selected as an experimental model for the enzyme study. Enzyme bioassay thus could remain useful technique to study sub lethal effects of drugs and toxic compounds.

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. Several platinum compounds were found to have antitumor properties against murine tumors and the most effective was cisplatin [6], Jorden *et al.*, [7] reported that anticancer drugs, Cisplatin and 5-fluorouracil inhibit ribosomal RNA in vivo.

5-Fluorouracil (5-FU) is also one of the most important anticancer drugs. In 5-fluorouracil, the hydrogen atom of the 5^{th} position of uracil is replaced by fluorine atom, 5-FU was designed to occupy the active sites ofthe desired enzyme targets, and thus inhibiting metabolic pathway in cancer cells. 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 [8]. All enzymes are chemically proteins in nature and control various sub cellular functions.

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 [9] suggested that the lysosomal enzymes get involved in many metabolic transformations *in vivo*.

The changes in acid and alkaline phosphatase activities in various organs of snails which serve as an intermediate host for trematode parasites have been reported by number of workers, Cheng [10] Karyakarte and Yadav [11] Krishna [12]. The increased rate of activities of acid and alkaline phosphatase is quite obvious in animals under morbidity condition. Among crustaceans, the distribution of acid and alkaline phosphatase and rise in their activity in the hemolymph, hepatopancreas [13], cuticle [14] and gastrolith walls [15] have been observed.

Acid and alkaline phosphatase enzymes are responsible for transphosphorylation and play an important role in overall energy metabolism of an organism. Bendse and Karyakarte [16] studied acid phosphatase and alkaline phosphatase activities in hepatopancreas of trematode, *Melania tuberculata* on exposure to toxicant secreted by *Cercaria bengalensis.*

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 condition the alkaline and acid phosphatase activity increases. Chronic exposure to anticancer drugs, Cisplatin and 5-fluorouracil increased the acid and alkaline phosphatase activities in various tissues of fresh water bivalve, *Corbicula strietella* [17].

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 fresh water bivalves, ***Parreysia corrugata*** (M) 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, *Parreysia corrugata* were divided into three groups with equal number of animals. They were kept in separate aquarium for 30 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 (LC_{50}/10 value of 96 hours) of Cisplatin, 1.007 ppm and another group was treated by sub lethal concentration (LC_{50}/10 value of 96 hours) of 5- Fluorouracil, 4.078 ppm.

On 10^{th}, 20^{th} and 30^{th} 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 Disodium

phenyl 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 37^0C 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

Effect of sub lethal concentration of cisplatin (1.007 ppm) and 5-fluorouracil (4.078 ppm) on acid phosphatase activity was studied in tissues such as gonads, digestive glands, mantle and foot of fresh water bivalve, *Parreysia corrugata*. Acid phosphatase activities determined are given in the Table. The enzyme activities of acid phosphatase were 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 *Parreysia corrugata* on exposure to chronic dose of Cisplatin and 5-fluorouracil.

Sr. No.	Tissue	Exposure to	10 Days	20 Days	30 Days
1	Gonads	Control	13.67 ± 0.714	14.05 ± 1.848	13.55 ± 2.192
		Cisplatin (1.007 ppm)	16.41 ± 1.782* (+20.04)	17.90 ± 1.419* (+27.40)	19.64 ± 1.199* (+44.94)
		5-FU (4.078 ppm)	15.91 ± 2.683* (+16.39)	16.78 ± 0.633* (+15.25)	18.27 ± 0.951* (+34.83)
2	Digestive glands	Control	12.18 ± 1.328	12.31 ± 2.551	11.81 ± 1.572
		Cisplatin (1.007 ppm)	13.80 ± 1.044* (+13.30)	16.28 ± 1.822* (+32.25)	18.40±1.327*** (+55.80)
		5-FU (4.078 ppm)	12.43 ± 3.070* (+02.05)	14.67 ± 3.368* (+19.17)	17.77 ± 3.841* (+50.46)
3	Mantle	Control	6.09 ± 0.0979	6.22 ± 0.914	5.97 ± 2.062
		Cisplatin (1.007 ppm)	8.08 ± 0.102** (+32.68)	9.20 ± 1.735* (+47.90)	10.32 ± 0.714* (+72.80)
		5-FU (4.078 ppm)	7.46 ± 1.633* (+22.49)	8.45 ± 2.347* (+35.85)	9.82 ± 0.874* (+64.49)
4	Foot	Control	2.61 ± 2.081	2.73 ± 0.102	2.98 ± 2.258
		Cisplatin (1.007 ppm)	4.10 ± 0.619* (+57.08)	4.35±0.098*** (+59.36)	4.97 ± 4.130* (+66.84)
		5-FU (4.078 ppm)	2.98 ± 1.478* (+14.18)	3.60 ± 0.870* (+31.87)	4.85 ± 0.918* (+62.75)

1. Values are expressed in K.A. units /100 gm of wet tissue/hour at 37 ^{0}C.
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.

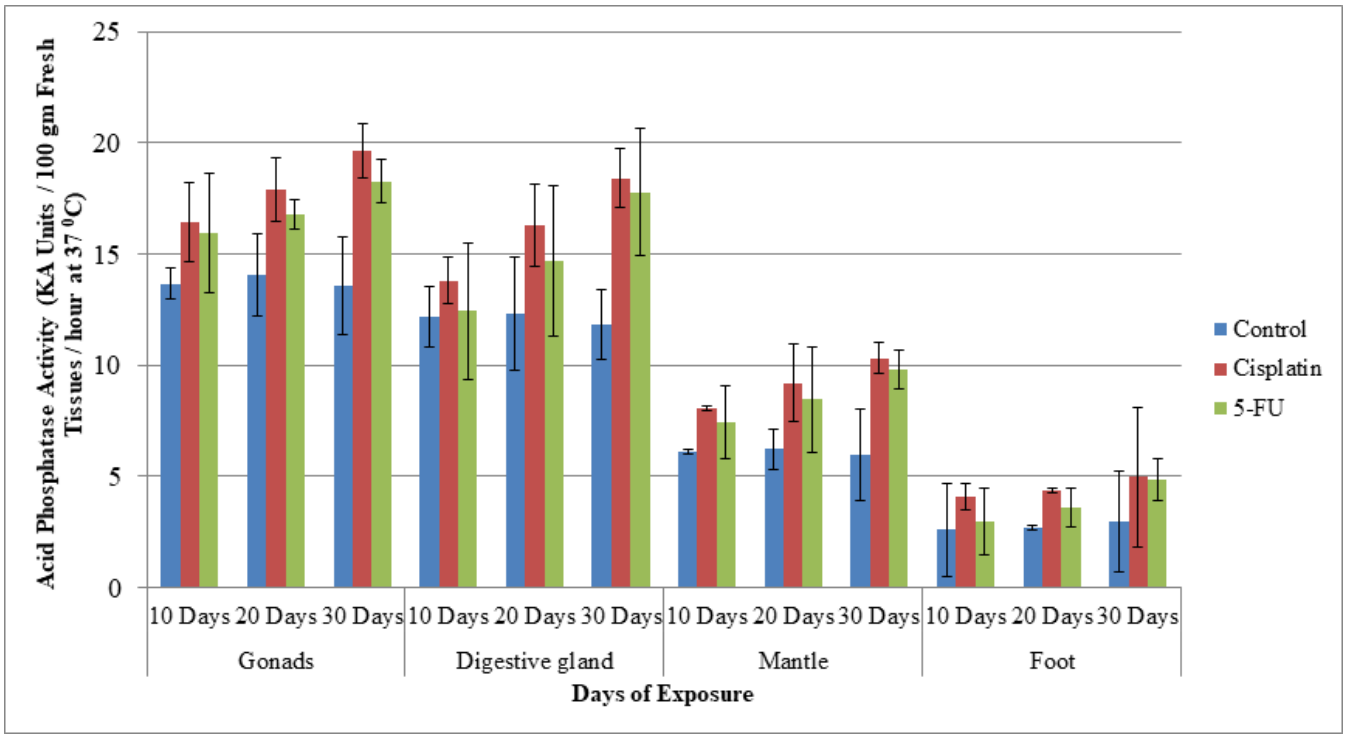

Fig. 1 : Acid phosphatase activity (K.A. units /100 gm of fresh tissue / hour at 37 ^{0}C) in different tissues of *Parreysia corrugata* after chronic exposure to Cisplatin and 5-Fluorouracil.

DISCUSSION:

Acid phosphatase is non-specific monoester. The acid phosphatase and alkaline 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 and 5-Fluorouracil was found to be increased in various tissues of *Parreysia corrugata* 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) and 5-fluorouracil (4.078 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, *Parreysia corrugata* 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 and 5-fluorouracil 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 activities of acid phosphatase and alkaline phosphatase on chronic exposure to anticancer drugs cisplatin and 5-fluorouracil was found to be increased in various tissues of fresh water bivalve, *Corbicula striatella* indicating the effect of the drugs on the cells with high metabolic rate [17].

Norseth [19] 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 [20]. 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 [21]. Ide and Fishman [9] suggested that the lysosomal enzymes cause

metabolic transformations in animals and leads into change in substrate specificity. 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 [22] Bhatia *et al.*, [23] declared that degradation and necrosis induced by toxicants in hepatopancreas leads to release of acid phosphatase enzyme. Dutta *et al.*, [24] concluded that the concentration of metals influences the induction and inhibition of phosphatase enzymes.

Increased acid phosphatase and alkaline phosphatase activity indicates the increased apoptosis and nucleic acid digestion in the Cisplatin and 5-Fluorouracil treated bivalves [17].

Increased acid phosphatase activities in various tissues of *Parreysia corrugata* indicate the increased apoptosis and nucleic acid digestion in the Cisplatin and 5-Fluorouracil treated bivalves.

CONCLUSIONS

1. Cisplatin and 5- Fluorouracil are used as anticancer drugs for the control of neoplastic growth. The effects of these anticancer drugs on the enzyme activity were studied on the experimental model animal fresh water bivalve, *Parreysia corrugata.*
2. The effect of chronic concentration (LC_{50}/10 value of 96 hours) of Cisplatin (1.007 ppm) and 5-fluorouracil (4.078 ppm) on acid phosphatase activity in gonads, digestive glands, mantle and foot of *Parreysia corrugata* was studied.
3. Acid phosphatase activity in gonads, digestive glands, mantle and foot of *Parreysia corrugata* were found to be increased significantly on chronic exposure to Cisplatin and 5- fluorouracil.
4. The Cisplatin and 5- fluorouracil on 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 *Parreysia corrugata.*
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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