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Effect of Actinomycin and 5-fluorouracil on Acid Phosphatase Activity of Gill, Gonad, Digestive Gland, and Foot Tissues of Fresh Water Bivalve, *Lamellidens Marginalis (lamark)*

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ABSTRACT

Actinomycin 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 Actinomycin and 5- fluorouracil (LC50/10 for 96 hours) were given to fresh water bivalves, Lamellidens Marginalis. For 45 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 Actinomycin treated bivalves than that of 5- fluorouracil treated bivalves.

KEYWORDS: *Anticancer drugs, Acid phosphatase, Cytotoxicity, Bivalves.*

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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. Therefore the freshwater bivalve, *Lamellidens Marginalis* is selected as an experimental model for the study. Enzyme bioassays remain however useful technique in looking for sub lethal effects of toxic effects of drugs and toxic compounds.Enzyme catalyzed reaction depends partly on how the enzyme and substrate bind together to form enzyme substrate complex will. The amount of which gives the rate of activity of the enzyme per unit time. An enzyme acid 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 actinomycin induced lymphoma toxicity has been attributed to reactive oxygen species (ROS) (Gulec M, et.al, 2004). ROS is a currently recognized mechanism in the pathogenesis of the actinomycin induced lymphoma toxicity in experimental study. (Atessahin A, et. al., 2006). Actinomycin causes lipid peroxidation (LPO) and decreases the activity of enzymes that protects against oxidative damage in immune system from actinomycin treated rats (Antunes LM, et. al., 2001). Oxidative damage caused by ROS has been implicated in the pathogenesis of actinomycin induced lymphoma injuries (Ilbey YO, et. al., 2009). Therefore, the fresh water bivalve, Lamellidens Marginalis 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. Thereby blocking metabolism in malignant cells. Although this antimetabolite is toxic, its efficacy makes it one of the most widely used agents against solid tumors (Francesco Pouci, 2008). The studies of enzymes create special interest because it lies just on the borderline where biological and physical sciences met. On the other hand, enzymes are of supreme importance in biology. Life depends on complex network of chemical reactions brought about by specific enzymes, and any modification of the enzyme pattern may have far-reaching consequences for the living organisms. All enzymes are proteins in nature and they control sub cellular functions. In the metabolism of protein, involvement of many enzymes, co-enzymes, intermediate proteins and amino acids are studied in many animals. All enzymes are chemically proteins in nature and control various sub cellular functions (Sekari K.G., et.al., 1968).

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. The increased rate of activities of acid phosphatase is quite obvious in animals under morbidity condition. Acid phosphatase enzymes are responsible for transphosphorylation and play an important role in overall energy metabolism of an organism. Bendse YD and Karyakarte PP. (1995) studied acid 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 acid phosphatase activity increases. Chronic exposure to anticancer drugs, Actinpmycin and 5- fluorouracil increased the acid activities in various tissues of fresh water bivalve, *Lamellidens Marginalis* (Bhosale PA., 2009). 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 selected experimental bivalves, *Lamellidens Marginalis* were collected from were collected from the kurla dam 5kms from Tq. Mahad. Dist. Raigad (M.S.). 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 4-5 days at 23^0C - 2823^0C 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 5.7-8.00 cm height X 6.6-7.3 cm length were selected from the aquarium and used for the experiments. The well acclimatized bivalves, *Lamellidens Marginalis* 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 actinomycin, 1.836 ppm and another group was treated by sub lethal concentration (LC50/10 value of 96 hours) of 5- Fluorouracil, 1.122 ppm. On 15th, 30th and 45th day of exposure, bivalves from each experimental group were dissected. The tissues such as gill, gonads, digestive glands, 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 (Gutman EB and Gutman AB., 1940). 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 370C for one hour. The reaction was terminated by adding 1 ml of Folin Ciocalteu's phenol reagent and reaction mixture was centrifuged at 6000 rpm for 05 minutes. Then 2 ml of 15 % sodium carbonate was added in each test tube of three repeats. The blue colour 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

Effect of sub lethal concentration of Actinomycin (1.836 ppm) and 5-fluorouracil (1.122 ppm) on acid phosphatase activity was studied in tissues such as gill gonads, digestive glands, and foot of fresh water bivalve, *Lamellidens Marginalis*. Acid phosphatase activities determined are given in the Table 1. The enzyme activities of acid phosphatase were expressed in KA units / 100 gram fresh tissue/hour at 370C. 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 1.

Table 1: Acid phosphatase activity in different tissues of *Lamellidens Marginalis* on exposure to chronic dose of Actinomycin and 5-fluorouracil

Sr. No.	Tissues	Exposure to	15 days	30 days	45 days
1)	Gill	Control	15.70±0.845	16.20±1.945	15.43±2.341
		Actinomycin (1.836 ppm.)	18.50±1.930* (+22.06)	19.20±1.816* (+26.27)	21.43±2.201* (+46.52)
		5-Fluorouracil (1.122 ppm.)	16.25±1587* (+20.07)	16.30±1.556* (+24.23)	19.25±2.451* (+42.25)
2	Gonad	Control	14.48±1.845	14.20±2.489	13.43±.1.214
		Actinomycin (1.836 ppm.)	15.15±1.987* (+18.45)	18.20±1.947* (+15.65)	20.43±2.487*** (+34.57)
		5-Fluorouracil (1.122 ppm.)	15.85±1.124* (+20.09)	17.20±1.124* (+23.27)	18.25±3.245* (+42.15)
3	Digestive gland	Control	8.09.23±0.845	8.20±1.945	07.43±2.341
		Actinomycin (1.836 ppm.)	10.21±0.458** (+34.46)	11.78±1.487* (+49.53)	12.74±0.28* (+74.71)
		5-Fluorouracil (1.122 ppm.)	9.25±1.564* (+21.32)	10.24±1.146* (+24.69)	8.43±2452* (+43.56)
4	Foot	Control	04.43±2.536	04.20±0.456	04.43±2.346
		Actinomycin (1.836 ppm.)	06.94±0.845* (+16.46)	06.20±0.945*** (+33.46)	06.43±4.341* (+64.46)
		5-Fluorouracil (1.122 ppm.)	5.20±1.124* (+15.29)	5.75±1.142* (+30.15)	6.00±2.456* (+41.23)

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

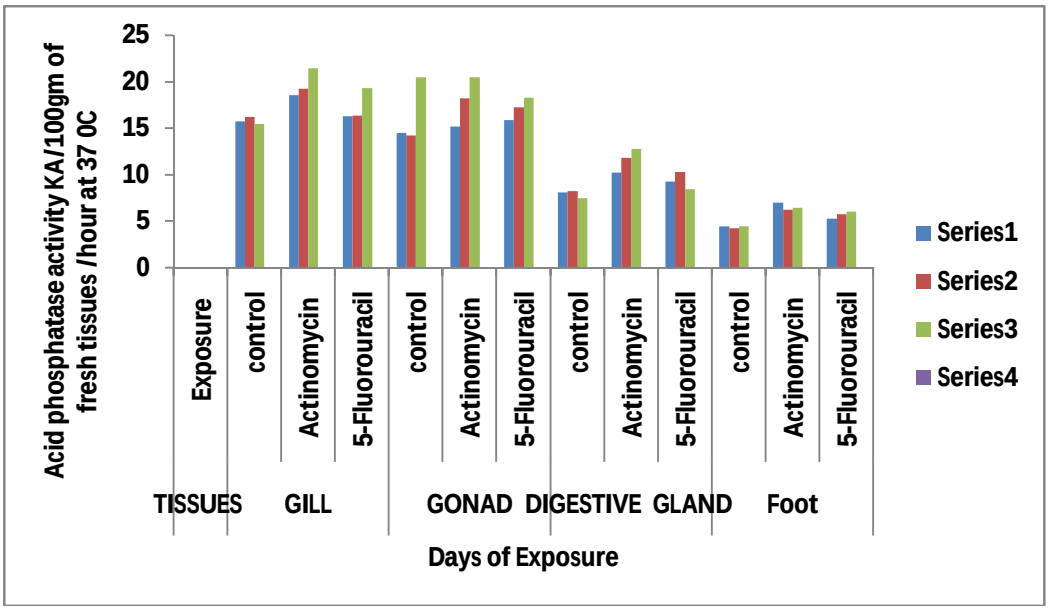

Figure 1: Acid phosphatase activity (K.A. units /100 gm of fresh tissue / hour at 37 ^{0}C) in different tissues of *Lamellidens Marginalis* after chronic exposure to Actinomycin and 5- Fluorouracil.

DISCUSSION

Acid phosphatase is non-specific monoester.In this present investigation it was observed that after chronic exposure toactinomycin (1.836 ppm) and 5- Fluorouracil (1.122 ppm), there was increase in the acid phosphatase activity in whole body and gonads of experimental bivalves, *Lamellidens Marginalis* as compared to those of control bivalves. As actinomycin and 5-Fluorouracil damages he nucleic acid specifically DNA, the cells becomes morbid and to recycle the phosphates the levels of these enzymes increases. In bone fracture cases, it is observed that the level of these enzymes increases in the serum. Also, the localization of ALP in the plasma membrane of rat hepatocytes changes following colchicine administration and bile duct ligation (Araki N., et. al., 1995).

Acid phosphatase, pre-eminently regarded as the marker enzyme, has been found in Golgi cisternae and lysosomes. Hiromu A., (1969) reported that acid phosphatase helps in the metabolism and transphosphorylation. Ide H. and Fischman W.H. (1969) suggested that the lysosomal enzymes undergo metabolic transformation in Vivo resulting in change of substrate specificity (Dutta H.S., et. al., 1983).
Concluded that both induction and inhibition of phosphatase taken place depending on the concentration of metals (Norseth T, 1967). Reported decrease in acid phosphatase activity due to accumulation of mercury in the lysosome and blockage in the release of enzyme. Generally, the increased activity of acid phosphatase was attributed to the activation of the enzyme, which was kept in a latent state inside the membrane of lysososmes, due to disruption of the concluded that sensitization of cell tissues may induce proliferation of smooth endoplasmic reticulum in hepatopancreas and resulted in increased production and liberation of acid phosphatase (Bhatia S.C. et.al., 1972), were of the opinion that degradation and necrosis induced by toxicants in hepatopancreas because release of acid phosphatase. Increased acid phosphatase activities in various tissues of *Lamellidens Marginalis* indicate the increased apoptosis and nucleic acid digestion in the Actinomycin and 5-Fluorouracil treated bivalves.

CONCLUSIONS

(1). Actinomycin 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, *Lamellidens Marginalis*.

(2). The effect of chronic concentration (LC50/10 value of 96 hours) of Actinomycin (1.836 ppm) and 5- fluorouracil (1.122 ppm) on acid phosphatase activity in gill, gonads, digestive glands, and foot of *Lamellidens Marginalis* was studied.

(3). Acid phosphatase activity in gill,gonads, digestive glands, and foot of *Lamellidens Marginalis* were found to be increased significantly on chronic exposure to Actinomycin and 5- fluorouracil.

(4). The Acinomycin and 5- fluorouracil on inhibiting the replication and transcription may induce the apoptosis and hence the activity of enzyme acid phosphatase increases in gill, gonads, digestive glands, and foot of *Lamellidens Marginalis*.

(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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