RESEARCH ARTICLE

IMPACT OF DOXORUBICIN ON ACID PHOSPHATASE ACTIVITY IN VARIOUS TISSUES OF THE FRESHWATER BIVALVE *LAMELLIDENS MARGINALIS*

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Abstract:

Doxorubicin is an anticancer drug that acts strongly and effectively in chemotherapy against solid tumors. This drug exhibits significant chemotherapeutic potential but also causes various biochemical alterations and cytotoxic effects in tissues. In the present study, sublethal doses of doxorubicin (LC_{50}/10, 96 h) were administered to the freshwater bivalve *Lamellidens marginalis* for 45 days. Acid phosphatase activity was estimated in different tissues of control and experimental bivalves using the method of Gutman and Gutman. The results revealed that acid phosphatase activity increased in various tissues with prolonged exposure to the anticancer drug in experimental bivalves. It was further observed that acid phosphatase levels were elevated in different tissues of doxorubicin-treated bivalves.

Keywords: Doxorubicin Toxicity, Acid Phosphatase Activity, Freshwater Bivalve, Lamellidens Marginalis, Tissue Enzyme Response, Cytotoxic Effects.

Introduction:

All enzymes are biological catalysts that regulate chemical reactions. They are chemically proteins produced specifically to carry out unique catalytic functions. Bivalve molluscs form an important part of aquatic biota, where anticancer drugs can enter the body and interfere with normal enzyme activity, leading to physiological and biochemical alterations. Freshwater organisms exposed to toxicants, even for short durations, may suffer severe damage to internal organs, particularly affecting enzymatic systems. Enzyme assays and metabolite estimations are considered reliable biochemical tools for monitoring toxicity caused by anticancer drugs. A normal regulatory mechanism always attempts to overcome inhibitory effects to maintain the overall health of the organism. Therefore, the freshwater bivalve *Lamellidens marginalis* was selected as an experimental model for enzyme studies. Early rubicin antitumor agents were discovered during studies on bacterial growth inhibition caused by

chemical complexes formed in experimental conditions. Although this antimetabolite is toxic, its effectiveness in chemoprevention makes it a widely used anticancer drug for treating solid tumors. All enzymes are chemically proteins in nature and regulate various subcellular functions (1, 2)

Acid Phosphatase:

Acid phosphatase is a nonspecific monoesterase, seemed as the biological marker enzyme. it has been observed in lysosome and Golgi cisternae. Acid phosphatase, a lysosomal enzyme, hydrolyses phosphate esters in acidic medium. The adjustments in acid phosphatase activity in diverse organs of snails which function an intermediate host for trematode parasites were said by means of wide variety of workers. Acid phosphatase enzymes are liable for transphosphorylation and play an essential position in normal energy metabolism of an organism (3).

Effect of Anticancer Drugs on Tissue Phosphatase Activity:

The influence of anticancer drugs on a series of physiological reactions can help to establish specific responses. High levels of toxic chemical compounds cause harmful effects on aquatic organisms. Anticancer drugs such as doxorubicin have been reported to increase acid phosphatase activity in various tissues of the freshwater bivalve *Corbicula striatella* (4). Therefore, these enzymes are used as diagnostic markers in scientific evaluation studies. The damaged RNA and DNA are also more susceptible to RNase and DNase attacks, respectively.

Methodology:

The fresh water bivalves, *Lamellidens marginalis* were collected from Kurla dam near Mahad, 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, *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 doxorubicin 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 (5). 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:

The effect of sublethal concentration of doxorubicin (1.007 ppm) on acid phosphatase activity was studied in various tissues such as gonads, digestive glands, mantle, and foot of the freshwater bivalve *Lamellidens marginalis*. The determined acid phosphatase activity is presented in the table. The enzyme activity was expressed in KA units per 100 g fresh tissue per hour at 37°C. Standard deviations were calculated from five replicates and are included in the table. The Student's *t*-test values and the percentage increase or decrease in enzyme activity are also presented in the table.

Table 1: Acid phosphatase activity in different tissues of , *Lamellidens marginalis* on exposure to chronic dose of Doxorubicin

Sr. No.	Tissues	Exposure	15 days	30 days	45 days
1	Gonads	Control	15.70 ± 0.845	16.20 ± 1.945	15.43 ± 2.341
		Doxorubicin (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
		Doxorubicin (0.836 ppm.)	15.15 ± 1.987* (+18.45)	18.20 ± 1.947* (+15.65)	20.43 ± 2.487*** (+34.57)
3	Mantle	Control	8.23 ± 0.845	8.20 ± 1.945	7.43 ± 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	4.43 ± 2.536	4.20 ± 0.456	4.43 ± 2.346
		Cisplatin (0.836 ppm.)	6.94 ± 0.845* (+16.46)	6.20 ± 0.945*** (+33.46)	6.43 ± 4.341* (+64.46)

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

Discussion:

Acid phosphatase is a nonspecific monoesterase and is widely recognized as a lysosomal marker enzyme involved in intracellular digestion and transphosphorylation reactions. Alterations in its

activity often reflect cellular stress and metabolic disturbance. In the present investigation, chronic exposure to cisplatin (1.007 ppm) resulted in a significant increase ($p < 0.05$) in acid phosphatase activity in the mantle, foot, gonads, and digestive glands of experimental bivalves compared with the control group. The elevation was more pronounced in gonads and digestive glands than in mantle and foot tissues, which may be attributed to their higher metabolic activity and greater sensitivity to toxic stress.

Cisplatin is known to induce nucleic acid damage, particularly DNA cross-linking, leading to cellular injury and activation of lysosomal enzymes to recycle degraded cellular components. The increased activity of acid phosphatase observed in the present study suggests enhanced lysosomal involvement in autophagic and apoptotic processes. Similar findings have been reported where toxicant exposure altered lysosomal enzyme activities due to membrane destabilization and release of hydrolytic enzymes (6). Norseth (7) reported a decrease in acid phosphatase activity following mercury bioaccumulation, attributed to enzyme inhibition within lysosomes. However, membrane damage may also cause enzyme leakage and increased measurable activity under induced stress conditions (8).

Acid phosphatase, localized in lysosomes and Golgi cisternae, plays a crucial role in cellular metabolism and phosphate turnover (9). Toxicant-induced sensitization of tissues may stimulate proliferation of smooth endoplasmic reticulum in digestive glands, leading to enhanced synthesis and release of lysosomal enzymes (10). The increased enzyme activity recorded in the present study therefore indicates enhanced apoptotic activity and nucleic acid degradation in drug-treated bivalves. Comparable increases in acid phosphatase activity in freshwater bivalves exposed to chemotherapeutic agents have been documented, suggesting that this enzyme serves as a reliable biomarker of cytotoxic stress (11).

Conclusion:

The effect of chronic concentration (LC_{50}/10 value of 96 hours) of cisplatin (1.007 ppm) on acid phosphatase activity in the gonads, digestive glands, mantle, and foot of *Lamellidens marginalis* was studied. The results showed that acid phosphatase activity in the gonads, digestive glands, mantle, and foot of *Corbicula striatella* increased significantly following chronic exposure to doxorubicin. Doxorubicin inhibits DNA replication and transcription, which may induce apoptosis, leading to enhanced lysosomal activity. Consequently, the activity of acid phosphatase increased in the gonads, digestive glands, mantle, and foot of *Lamellidens marginalis*, indicating cellular damage and metabolic stress caused by anticancer drug exposure.

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