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Effect of surfactants on phosphatase level of fresh water fish *Labeo rohita*

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Abstract: *Alterations in the activity of enzymes Acid phosphatase (E.C.3.1.3.2) and Alkaline phosphatase (EC 3.1.3.1) in organs such as liver, gills and muscle of rohu following its exposure to surfactants viz. CTAB, SDS and Triton X- 100 were analyzed. Different levels of exposure were given depending on the LC_{50} value of the surfactant used. Also, the influence of age and weight of the organisms was tested simultaneously. The activity of ACP in the tissues of fish exposed to all the three surfactants showed marked enhancement after exposure; the effect being highest in the liver followed by gill and muscle. Activity levels of ALP in different tissues of the fish exposed to the surfactants also showed an increase. Maximum increase was found in the liver followed by muscle, and gill. The increase in the levels of these enzymes indicates a stressful condition of the fish.*

Key words: *Surfactant, Phosphatase, Rohu*
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Introduction

Surfactants are the most widely used groups of compounds today, with wide application in industry and household. These are unique substances that contain hydrophobic and hydrophilic moieties within their molecule and find enormous applications in biology. However, many surfactants are toxic above certain concentrations and therefore their toxicity study is an important area of research. Cationic surfactants are commonly used as germicidal and antibacterial agents whereas anionic surfactants are used for laundry and cleaning purpose. Increased concentration of surfactants in water results in higher level of phosphates and nitrates hence the problem of eutrophication is very common in water bodies. In such cases luxuriant growth of algae is stimulated due to addition of nutrients in water.

Available toxicity data on surfactants largely comprise of works related to mortality, larval development and reproductive capacities (Rosety *et al.*, 2001; Zelimira *et al.*, 2005; Sandbacka *et al.*, 2000; Lewis *et al.,* 1995). Surfactants facilitate dissolution of carcinogenic substances such as polycyclic aromatic hydrocarbons (PAH) which are otherwise insoluble in water (Ledakowicz *et al.*, 2002). They also cause harm to various biomolecules related to the metabolic processes. Enzymes are one of them to control the metabolic processes.

Acid phosphatase (ACP E.C.3.1.3.2) is hydrolytic in nature and helps in the autolysis of the cell after its death (Novicoff *et al.*, 1961). It could be used as indicator for studying cell mortality due to intoxication. Alkaline phosphatase (ALP E.C.3.1.3.1) is also responsible for transphosphorylation. The study of the activity levels of ALP and ACP was carried out to measure the effect on metabolic activity by observing variation in enzyme levels after exposure to surfactants.

Three different surfactants namely cetyltrimethylammonium bromide (CTAB) a cationic surfactant, sodium dodecyl sulphate (SDS) an anionic surfactant and Triton X- 100, a non-ionic surfactant were chosen for the present study. Though these do not play a direct role as prominent detergent the objectives were to evaluate the effects of a single exposure of CTAB, SDS and Triton X- 100 for different periods of observations with reference to change in the activity levels of ACP and ALP. Fish rohu is chosen as it is readily available through out the year easy to maintain, convenient for testing and sensitivity to chemicals.

Materials and Methods

Experimental conditions: 100 l capacity PVC lined glass aquaria equipped with continuous air supply was stocked with 10 rohu fingerlings in each. The basic physico-chemical parameters *viz.* dissolved oxygen, pH, alkalinity and total hardness were measured systematically and its optimal level was analyzed and maintained as prescribed by APHA (1995) (dissolved Oxygen 7.4 ± 0.2 mg l^{-1}, pH 8.3 ± 1,total alkalinity 118-132 mg $CaCO_3$ per litre, total hardness 122-136 mg $CaCO_3$ l^{-1}). Fish were fed with the food fortified with vitamin (rice bran: oilcake 2:1) @ 5% of their body weight, once daily through out the experimental period. Water was changed along with waste feed and faecal materials every 48 hr and the water temperature during the experiment was maintained 25±5°C. The fish were divided in to 10 groups randomly for conducting the assay. Each group had 10 fingerlings (weight 6 to 8 g) maintained in aquaria.

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Preparation of surfactant solutions and administration: CTAB, SDS and Triton X- 100 (Fluka, Switzerland) were used with all precautionary measures. Stock solutions were prepared using tap water (50 mg ml^{-1} *i.e.* 50000 ppm) and stored at 4°C. Known concentrations of the surfactants were then prepared by adding stock solution to a measured quantity of water in experimental aquaria. Known levels of surfactants for subchronic trials were selected based on the (a) LC_{50} value of rohu fingerlings to CTAB, SDS and Triton X-100 (10, 100 and 100 ppm) (b) age and weight influence of the fingerlings. The fish were exposed to 1.00 and 10 ppm of SDS, 0.1 and 0.5 ppm of CTAB, 1 and 5 ppm of Triton X-100 respectively. The experimental animals were exposed to the surfactant for a period of 10 and 20 days respectively. The control group of fish was exposed for the same period in chlorine free tap water, in controlled physicochemical conditions.

Enzyme methodology: To study the activity levels of enzymes in the tissues of various groups of exposed fishes, they were dissected at temperature 1-5°C. Their organs $tissues^{-1}$ removed and immediately homogenized in 0.025% sucrose (chilled) solution. The homogenates were directly used for the enzyme assays using colorimeter. (Andersch *et al.*, 1965; Bessey *et al.*, 1965).

Results and Discussion

The approach of this study allows a systematic investigation of the effects of surfactants at enzymatic levels. The activity of ACP in the tissues of fishes exposed to all the three surfactants showed marked enhancement after the exposure. The rate of enhancement was highest in the liver followed by gill and muscles. The effect of surfactants on specific activity of ACP in liver is shown in (Fig. 1). In liver exposed to higher concentration of TX-100 the activity levels of ACP ($p<0.05$) significantly increased from the normal activity level. Similar trend was observed for the low concentration of TX-100 ($p<0.05$). On exposure to high concentration of CTAB for a period of 10 days, even though there was an increase in ACP level it was not highly significant in lower as well as higher concentrations. When exposed to *SDS*, the activity levels of ACP showed least increase in both the concentrations ($p<0.05$).

In the gill exposed to both high and low concentrations of TX-100, maximum increase in activity levels of ACP was observed ($p<0.05$). While exposure to both high and low concentrations of CTAB and SDS elevated the activity to a negligible extents (Fig. 2).

In muscles exposed to high/low concentration of TX-100, the level of activity increased slightly after 10 and 20 days though the change was not significant ($p>0.05$) (Fig. 3). Changes in the activity levels of ACP in muscles of fishes exposed to CTAB and SDS were not significant ($p>0.05$). Activity levels of ALP in different tissues of the fishes exposed to the surfactants showed increase in the activity. The maximum increase was found in the liver exposed to TX-100, followed by muscles and gills.

Liver exposed to both, high and low concentrations of TX-100, activity levels were increased enormously after 20 days from

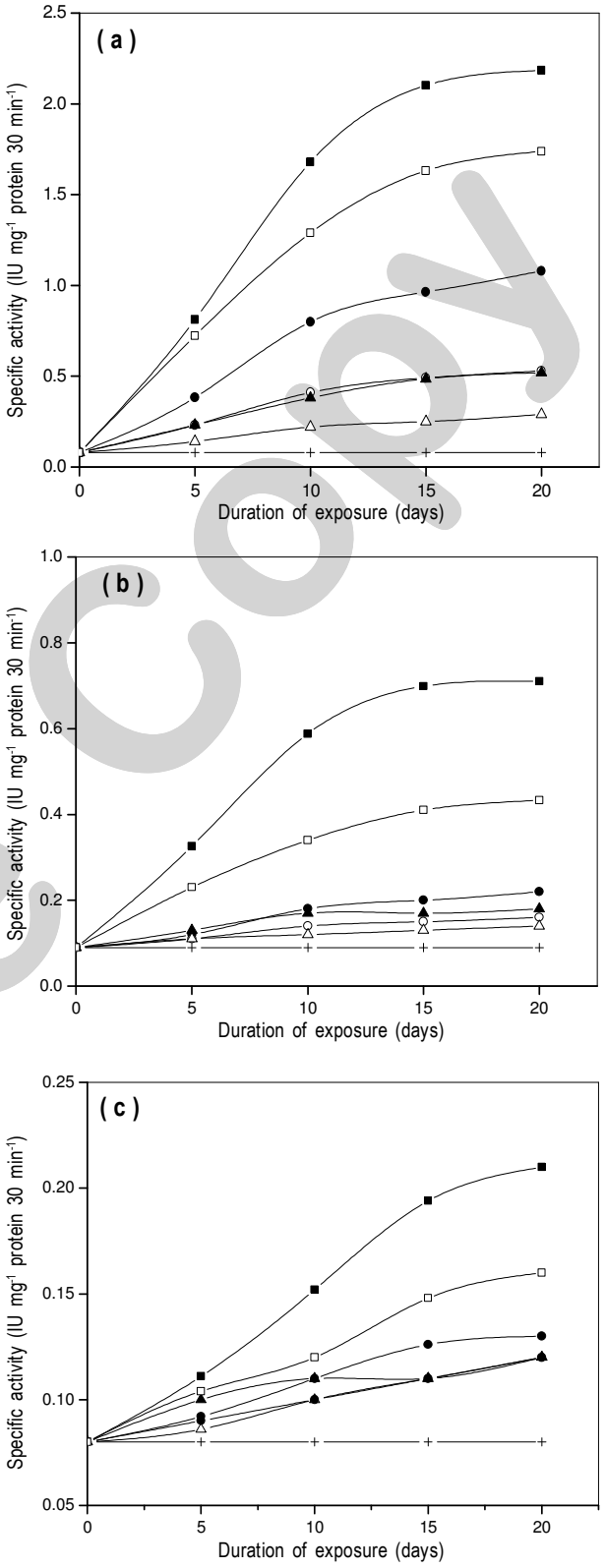

Fig. 1: Effects of surfactant concentrations on the specific (a) liver (b) gill and (c) activity of acid phosphatase in muscle ● and ○ CTAB, ▲ and △ SDS, ■ and □ TX 100 + control (open symbols = Low concentration, Solid symbols = High concentration)

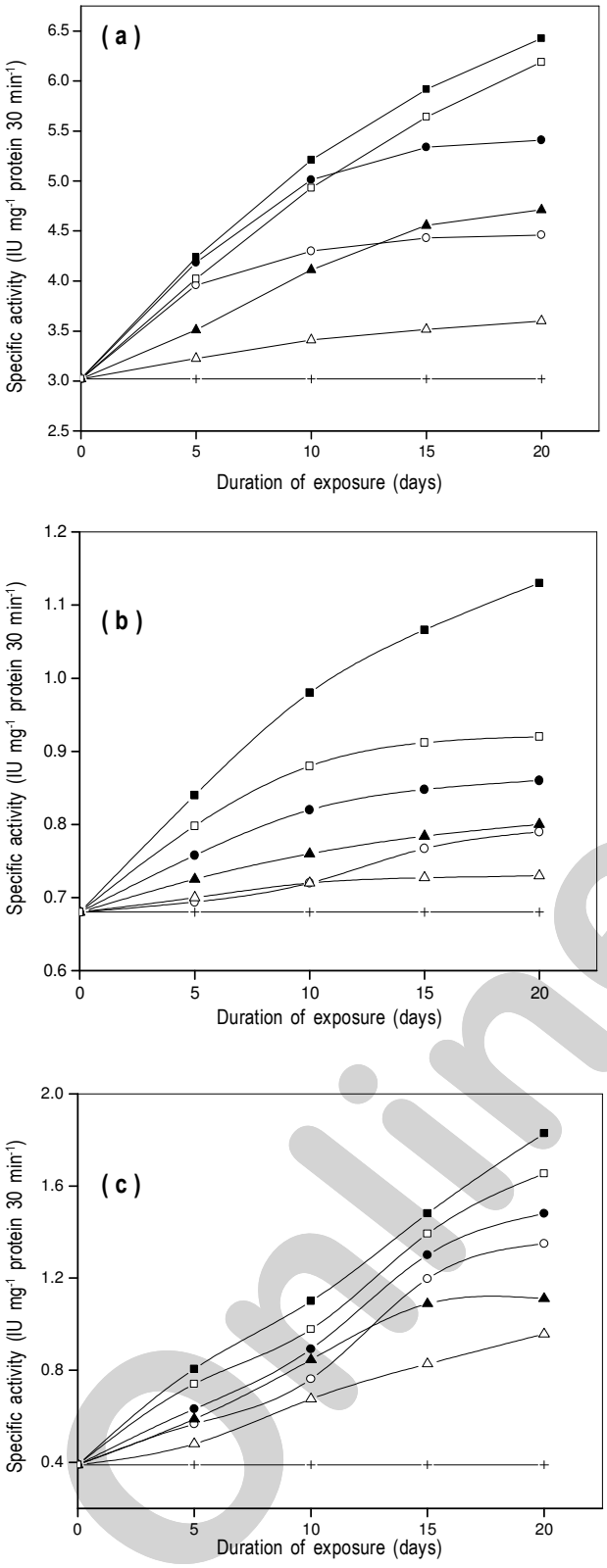

Fig. 2: Effects of surfactant concentrations on the specific activity of in (a) liver, (b) gill and (c) of alkaline phosphatase in muscle ● and ○ CTAB, ▲ and △ SDS, ■ and □ TX 100 + control (open symbols = Low concentration, Solid symbols = High concentration)

the normal level. Liver exposed to both, high and low, concentrations of CTAB and SDS also showed a remarkable elevation in the activity levels of ALP (Fig. 4). A similar pattern of increase followed in all the other tissue viz .muscle and gill exposed to all the three surfactants though the increase was not as remarkable as in liver (Fig. 5,6). The gill may be the primary target for surfactants and many other toxicants in the water due to its large surface area and thin epithelium. A significant increase in liver and muscle ACP and ALP was noted earliar in DEP (diethyl pthalate) treated fresh water fish *Cirrhinus mrigala* due to increased lysosomal activity in liver and muscle (Ghorpade *et al.*, 2002). Such an increase in ACP and ALP level in rat treated with DEP in has also been reported earlier in rat by (Sonde *et al.*, 2000) for studying cell intoxication, its death and they can be used as indicators the increase in ACP level in liver and gills may be due to increased lysosomal activity.

The activity level of ALP was observed to increase after exposure to anionic surfactants. During the treatment of TX-100 to the brush border cells of intestine, a sharp elevation in the activity of ALP was measured and later on a decline was observed with increase in surfactant concentration. ALP is a brush border enzyme which splits phosphate esters in an alkaline pH and mediates membrane transport (Goldfisher *et al.*, 1964).

This way it controls the cellular energetics by the process of transphosphorylation and as surfactants can solubilize membranes, the study of the activity levels of ALP can be revealing.

In conclusion, sublethal concentrations of surfactants have direct effect on phosphatase activity in *Labeo rohita*. The activity of ACP in the tissues of fish exposed to all the three surfactants showed marked enhancement after exposure. The rate of enhancement was highest in the liver followed by gill and muscles. Activity levels of ALP in different tissues of fish exposed to the surfactants also increased. The maximum increase was found in the liver of fish exposed to TX-100, followed by muscles and gills of the same surfactant.

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