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Subchronic Toxicity of Ionic Surfactants on Freshwater Fish *Labeo rohita* (Hamilton)

R.P. PATEL, D. MANDAL and A. BAHADUR*

Department of Zoology
P.T.S. College of Science
Surat 395001 India

Abstract

Histological and histopathological alterations in different organs such as liver, gills, kidney, intestine and brain of *Labeo rohita* following its exposure to two ionic surfactants, cetyltrimethylammonium bromide (CTAB) and sodium dodecyl sulphate (SDS), were investigated. Different levels of exposure were given depending on the LC_{50} value of the surfactants used. The influence of age and weight of the organisms were tested simultaneously. The visual and microscopic results of organs were time and concentration dependent. When exposed to 10 ppm surfactant, liver hepatocytes showed fatty deposition and distortion in their arrangement and gills showed distortion and degeneration of epithelial cells. The groups exposed to different doses of SDS showed the toxic effects viz. necrotic changes visible in tissue in lower concentration and slight congestion of brain and inflammatory changes in the intestine. The exposure to CTAB in fishes revealed necrotic and vascular changes in liver, massive degeneration of epithelial cells in gills, congestion in brain, inflammatory changes in intestine and rupture of mucosa. During exposure to very low doses of surfactants, fish did not show any mortality but behavioural changes were observed. Cationic surfactant CTAB was more toxic to fish than the anionic SDS and it affects tissues involved in gaseous and nutrient exchange more severely.

* Corresponding author. Tel.: +91 98 2545 4366
E-mail address: anita26p@gmail.com

Introduction

Surface active agents (surfactants) are versatile compounds with wide applications in industry and household. These have unique structure that contain hydrophobic and hydrophilic moieties within their molecule and find enormous applications in biology viz. in mimicking enzyme catalysed reactions and biomembranes and many others. However many surfactants are toxic above certain concentrations and therefore toxicity of surfactants is an important area of research.

The consumption of surfactants is increasing and they are discharged into the sewage system with many undesirable phenomena associated including a destructive action on fauna and flora of surface waters and many cause eutrophication in higher concentrations. Available toxicity data on surfactants largely comprise of works related to mortality, larval development and reproductive capacities (Moffet and Grosch 1967; Swedmark et al. 1971; Abel 1974; Lewis 1991; Chattopadhyay and Konar 1996; Katherine et al. 2003) but reports are very few on deleterious effects of surfactants on tissues and organs of fish. Surfactants facilitate dissolution of carcinogenic substances such as PAH (polycyclic aromatic hydrocarbons) which are otherwise insoluble in water (Ledakowicz et al. 2002). Their concentrations in natural environment like river water and sediments range between 1-10 ppm for sodium alkyl sulphate and 5-50µg•L^{-1} for cationic (Boethling and Lynch 1992; Painter 1992).

Indian major carp (*Labeo rohita*), the prime cultured species in India occupies a prominent position in the aquatic system, hence the importance of surfactants on the cellular level of the species was chosen for study. The present study has been undertaken with a view to find the pathological effects of ionic surfactants in rohu (*Labeo rohita*). Till date the susceptibility of this species to ionic surfactants is unknown. Two ionic surfactants namely CTAB (cetyltrimethylammonium bromide) a cationic surfactant and SDS (sodium dodecyl sulphate an anionic surfactant) were chosen for the present study. The objectives were to evaluate the effects of a single exposure of the surfactants for different periods at the tissue level. This was conducted repeatedly for a series of concentration.

Materials and Methods

Experimental conditions

One hundred (100) L capacity PVC lined glass aquaria equipped with continuous air supply were stocked with 10 *Labeo rohita* fingerlings in each. The basic physicochemical parameters viz. dissolved oxygen, pH, alkalinity and total hardness were measured systematically and its optimal level was maintained according to APHA (1995) (dissolved Oxygen 7.4 ± 0.2 mg.L^{-1}, pH 8.3 ± 1). Fish were acclimatized to laboratory condition in well aerated condition and fed with rice bran oilcake 2:1 fortified with vitamin at 5 % of their body weight, once daily throughout the experimental period. Water along with waste feed and faecal materials was changed every 48 hours and the water temperature during the experiment was maintained 25 ± 5°C. The fishes were divided in to 10 groups randomly for conducting the assay. An average of four fish for a particular set of experiment was always taken.

Preparation and administration of surfactant solutions

CTAB and SDS (Fluka, Switzerland) were used with all precautionary measures using necessary equipments. Stock solutions were prepared using tap water (50mg•ml^{-1} i.e. 50000 ppm) and stored at 4°C. Desired concentrations of the surfactants were then prepared by adding stock solution to a measured quantity of water in experimental aquaria (APHA 1995).

Different levels of surfactants for subchronic trials were selected based on the (a) LC_{50} value of fingerlings to CTAB, and SDS respectively (b) age and weight influence of the fingerlings. The fish were exposed to, 1.00 and 10 ppm of SDS and 0.1 and 0.5 ppm CTAB. Fish were exposed to the surfactant for a period of 10 days or 20 days. The control group of fishes were exposed for the same period in chlorine free tap water, in controlled physicochemical conditions.

Pathological and mortality analysis

Number of mortality with both the surfactants was noted and liver, kidney, intestine, gills and brain of dead fish were visually examined for gross lesions. Behavioural changes during the trial were also noted. Fish were randomly selected from both the subchronic trial, at the end of the experimental period and examined externally for gross lesions. Selected fish were dissected and different organs were fixed in Bouins fluid for 12

hours for histological examination. The tissues were washed in running tap water for 12 hours and were dehydrated and infiltrated with wax. Semithin sections (4-5 µm.) from paraffin block were taken and stained with Ehrlich's Haematoxylin and Eosin for light microscopy (Denza and Barbara 1980; Drury and Wallington 1980). Stained sectioned were examined and photographed under a compound microscope fitted with a charge coupled device (CCD).

Results

Behavioural and external change

Fish exposed to ionic surfactants showed anorectic changes and showed sluggish behaviour in general with intermittent hyperactive movements. The most observed behavioural changes were loss of equilibrium, rapid gill movement and staying motionless at the bottom of the aquarium. High mucous secretion was seen in all fishes exposed to the surfactants (Table 1). An interesting finding that was observed is that skulls of all the fish became translucent making brain visible. Such a change could probably occur due to the decalcification. Further studies are being carried out to come to a definite conclusion.

Table 1. Exposure and effects of surfactants: Behavioural and external changes

Surfactant	Concentration (ppm)	Exposure Period	Effects
SDS	1	10-20 days	High mucous secretion
	10	10-20 days	Showed distress initially but seemed acclimatised after 48 hours
CTAB	0.1	10-20 days	Rapid opercular movements observed
	0.5	10-20 days	Became very sluggish after 24 hrs and food consumption became erratic and suffered from severe diarrhoea

Pathological changes and mortality

In the subchronic trials a few fish had whitish gills and showed severe haemorrhage in the region surrounding gill. On necropsy, the colour of liver was pale yellow to light red in a few cases. In some cases the liver had areas with blood accumulation and abdominal fat was noted. Brains of

a few fishes showed cerebral congestion and haemorrhage in the cranial cavity. Peritoneal adhesions of abdominal organs were seen in some fishes. Intestine of all the fishes were filled with mucoid exudates and this was severe in fishes treated with CTAB. Kidney of fish exposed to 10ppm SDS for 20 days showed mild haemorrhage. There were no gross lesions in fish of control group.

Histopathological changes

The lesions observed during the sub chronic trials were dose and time dependent. Major changes were seen mostly in the gills, liver, brain, intestine and kidney. Gill lamella showed necrosis at places in both the concentrations of the surfactants. The rupture of gill lamellae at the extremities and lateral margins causing haemorrhage in a few fishes exposed to higher concentration of CTAB was observed. Adhesion and fusion of secondary gill lamellae were also quite prominent at some places. Erosion of gill lamella was observed at places (Fig. 1). In liver, the hepatocytes showed massive necrosis and fat like deposition was seen at places in both the experimental groups. Vacuolization and hydropic changes were seen. Residual bodies like structures were reported in the cytoplasm of hepatocytes (Fig. 2).

The brain showed generalized congestion and infiltration of mononuclear cells in the meninges and general disruption of cellular structure of the six layers of the optic tectum was observed (Fig. 3). There was partial sloughing of mucosa into lumen in intestines of fishes at exposure to higher concentrations of both the surfactants. Tissue loosening in the mucosa of the intestine was also observed (Fig. 4). Kidney tubules showed dilation in the lumen when exposed to SDS and constriction of lumen when exposed to CTAB showing antagonistic properties. A peripheral space developed outside the Bowman's capsule due to shrinkage when treated with CTAB and swelling of Bowman's capsule was seen during exposure to SDS (Fig. 5).

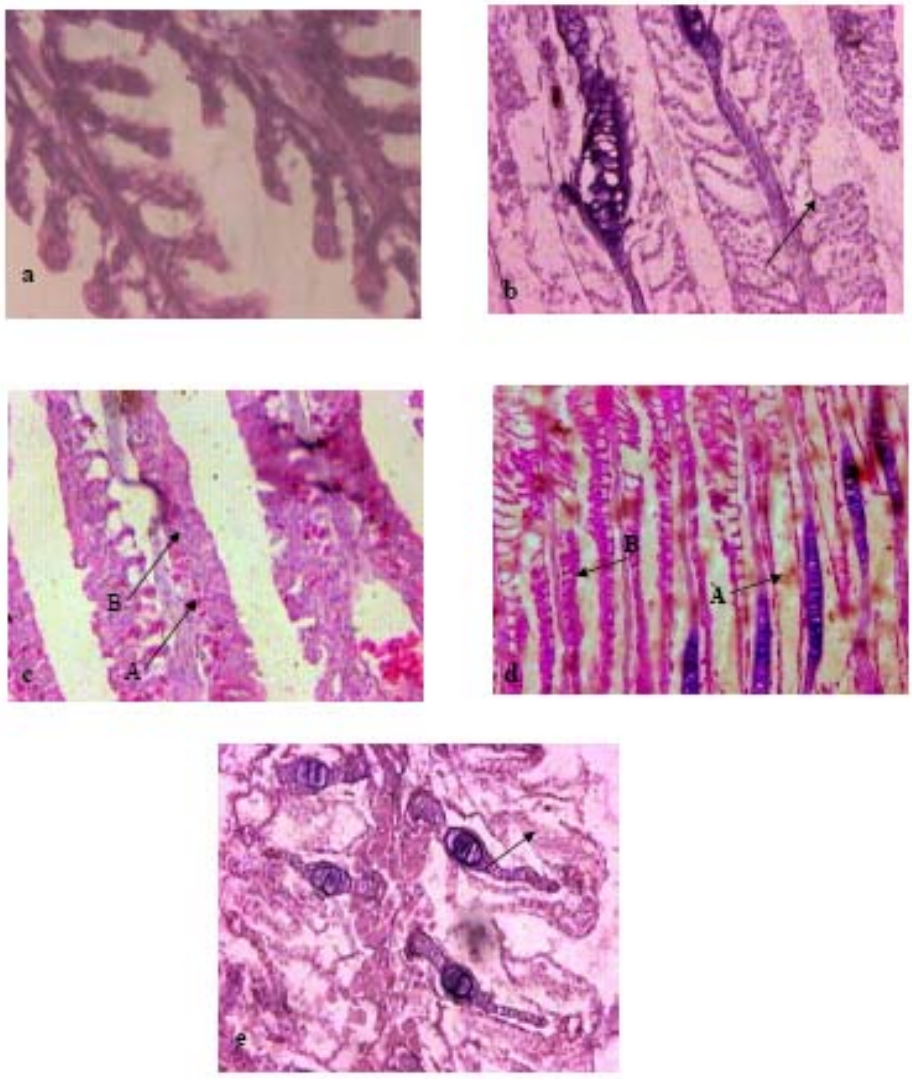

Figure 1. Histology of gills: (a) Normal gills, (b) SDS 10 ppm exposed for a period of 10 days, (c) SDS 10 ppm exposed for a period of 20 days, (d) CTAB 5 ppm exposed for a period of 10 days, (e) CTAB 5 ppm exposed for 20 days

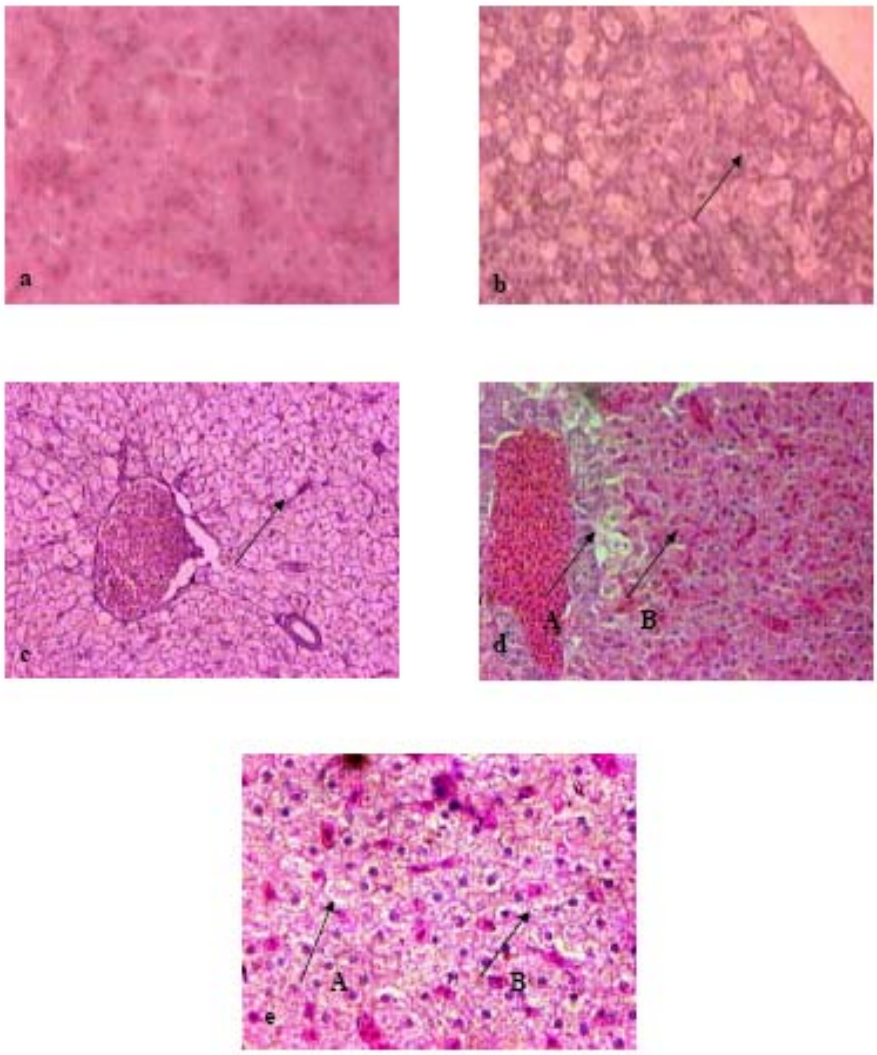

Figure 2. Histology of Liver: (a) Normal liver, (b) SDS 10 ppm exposed for a period of 10 days, (c) SDS 10 ppm exposed for a period of 20 days, (d) CTAB 1 ppm exposed for a period of 20 days, (e) CTAB 5 ppm exposed for a period of 10 days

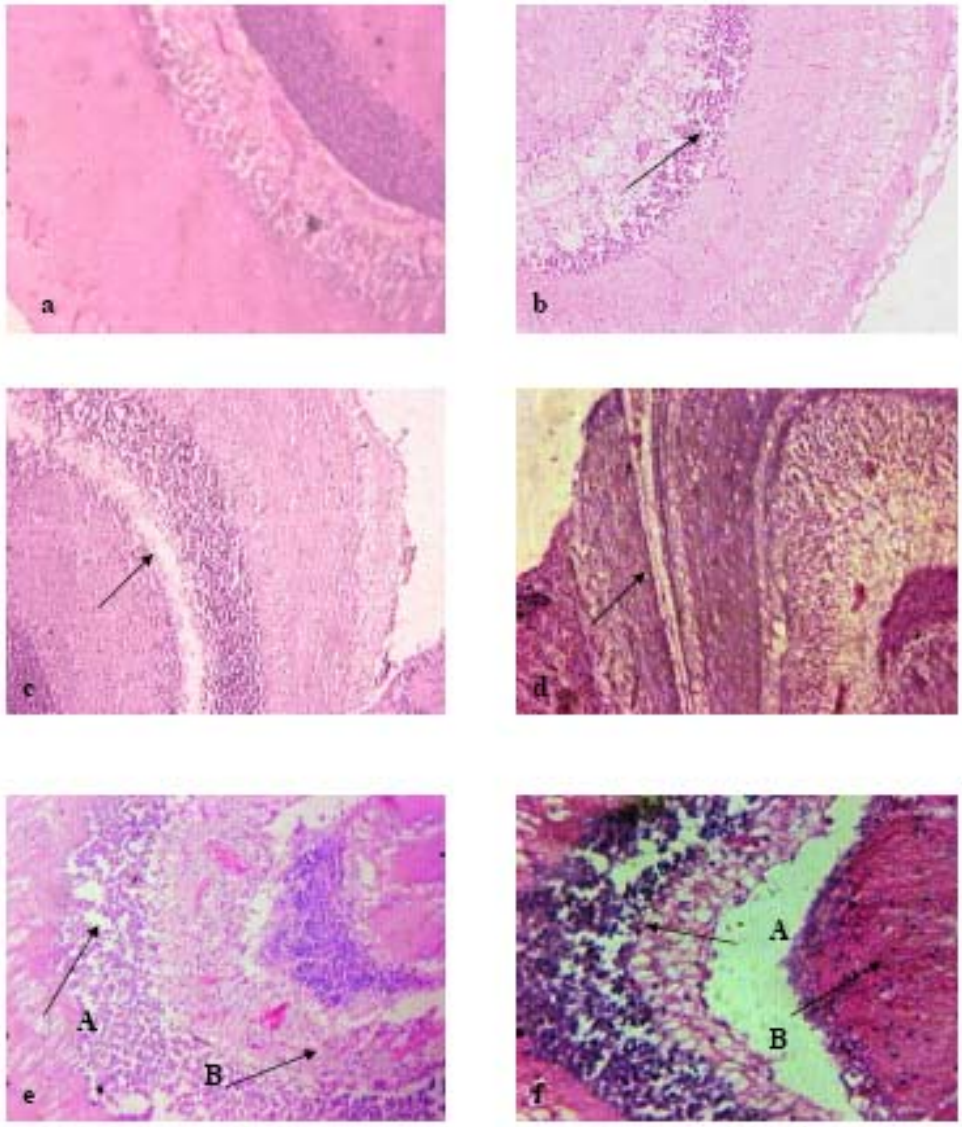

Figure 3. Histology of Brain: (a) Normal brain, (b) SDS 1 ppm exposed for a period of 10 days, (c) SDS 10 ppm exposed for a period of 10 days, (d) SDS 10 ppm exposed for a period of 20 days, (e) CTAB 1 ppm exposed for a period of 20 days, (f) CTAB 5 ppm exposed for a period of 10 days

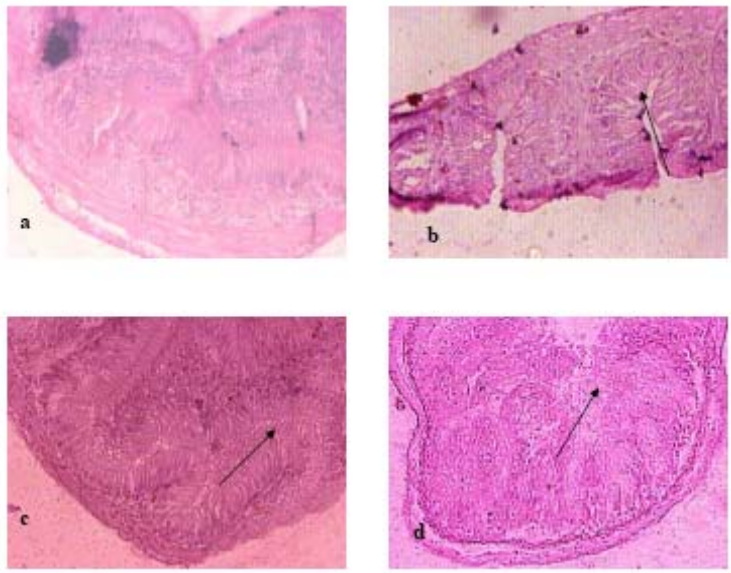

Figure 4. Histology of Intestine: (a) Normal intestine, (b) CTAB 5 ppm exposed for a period of 20 days, (c) SDS 10 ppm exposed for a period of 10 days, (d) SDS 10 ppm exposed for a period of 20 days

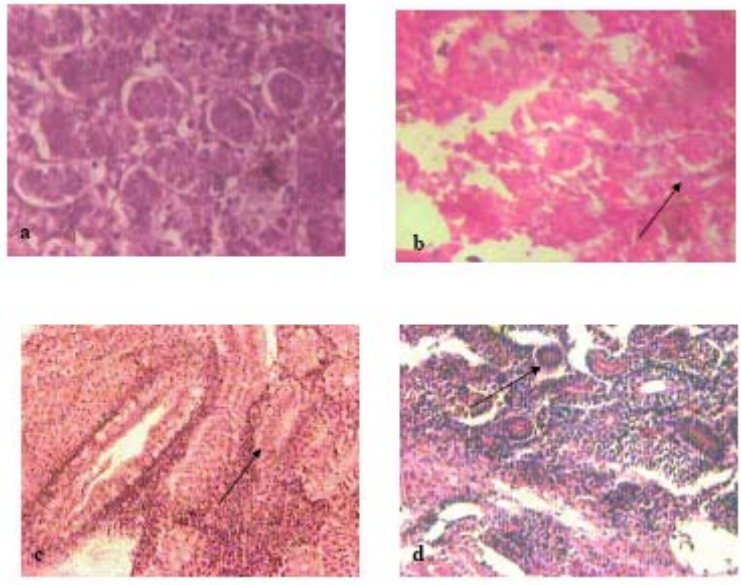

Figure 5. Histology of Kidney: (a) Normal kidney, (b) SDS 1 ppm exposed for a period of 10 days, (c) SDS 1 ppm exposed for a period of 20 days, (d) CTAB 5 ppm exposed for a period of 10 days

Discussion

In the present study the erratic swimming movement and anorexic changes seen in fish can be a result of toxicosis. Similar results have been reported in labeo treated with aflatoxin by Sahoo et al. (2001). Histological studies clearly reveal the marked congestion in most organs and are clear indications of toxic effects. Rapid opercular movement can be taken as an index of the stress felt by the fish. It is likely that high secretion of mucus may help in protecting vital organs, like gills and intestine against surfactant toxicity. The gills showed marked alterations in the epithelia. There was fusion in adjacent secondary lamellae resulting in hyperplasia, with profound oedematous changes, characterized by epithelial detachment. In the liver, the enlargement of the hepatocytes was related to the concentration and duration of exposure to glyphosate (Sahoo et al. 2003; Olurin et al. 2006).

Liver is a primary organ for detoxification of xenobiotics. Narayan and Singh (1991) observed extensive degeneration of cytoplasm with pyknosis of nuclei in liver of *Heteropneustis fossilis* while subjecting them to acute thiodan toxicity. Similar changes were recorded in the present study with ionic surfactants The destruction of cellular arrangement, fat like deposition and residual bodies in hepatocytes in fish exposed to 0.5 ppm CTAB for 20 days can be an effort by the fish to eliminate the surfactant. In the liver, the enlargement of the hepatocytes was related to the concentration and duration of exposure. Large vacuoles in the hepatocytes were also seen by Olurin et al. (2006).

The histopathological changes by HCH have been studied by Das and Mukherjee (2000) in *Labeo.* The HCH is found by them to be neurotoxic causing vacuolisation of brain parenchyma and moderate swelling of the pyramidal cells of cerebrum. Similar observation was seen in this study. Cationic surfactant was found to be more toxic to fish than the anionic surfactant and tend to affect the tissues involved in gaseous and nutrient exchange more severely. Cationic surfactants have been observed to be toxic in many other vertebrates (Jungermann 1970).

Being cationic CTAB has more affinity for negatively charged membrane lipids and they are not readily metabolized. The SDS on the contrary is readily biodegradable and induces increase in antioxidant glutathionin, which probably helps the animals to overcome the toxic stress to a large extent (Olurin et al. 2006).

Conclusion

Results of the present study indicated that *Labeo rohita* is susceptible to ionic surfactant and even concentrations of 1.00 and 10 ppm of SDS and 0.1 and 0.5 ppm of CTAB were sufficient to produce significant lesions in body tissue. The typical histopathological changes observed in the present study can be regarded as diagnostic aid for fish exposed to surfactants. *Labeo* can also serve as an effective bio-monitor for surfactant induced toxicopathic stress (Jifa et al. 2005).

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