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Toxic Effects of Sodium Dodecyl Sulphate on Grass Carp *Ctenopharyngodon idella*

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Sodium dodecyl sulphate, an anionic detergent finds various applications in household, industrial, laboratory and pharmaceutical preparations.This paper reports the histological, enzymatic and microbial changes found in the gills of Grass Carp (*Ctenopharyngodon idella*) induced by the acute action of Sodium dodecyl sulfate (SDS). A 96 h LC_{50} value of 7.7 mg/l of SDS was obtained using static bioassay method. Sets of twenty grass carps each were exposed to nominal concentrations (2, 2.5 and 3 mg/l) of SDS for 30 days and the changes in the gills were monitored every tenth day. Extensive gill damage with necrosis and intraepithelial oedema was observed. This was accompanied by increased levels of antioxidant enzymes *viz*., superoxide dismutase and catalase. The gills of SDS treated fishes also showed an increased rate of microbial attack predominantly by *Aeromonas* sp, *Micrococcus* sp and *Bacillus* sp. Extensive necrosis, and increased rate of microbial attack suggested that SDS incites an acute inflammatory response. The morpho-histochemical changes in the gills provoked functional disorders *viz*., asphyxia and loss of osmotic and ionic regulation that may ultimately play a significant role in the mortality of Grass Carp exposed to SDS.

Key words: Sodium dodecyl sulphate, *Ctenopharyngodon idella*, catalase, superoxide dismutase.

The increased disposal of xenobiotics like hydrocarbons and pesticides has aroused much concern on their ecological impact. Most often, ecological impact of synthetic detergents are taken lightly when compared to other xenobiotics. Once used these detergents find their way into waterbodies, where they can cause adverse effects if they persist for long periods, leading to accumulation of potentially toxic or otherwise harmful substances (Tiehm, 1994; Deschenes *et al*., 1996).

Of the many available detergents, SDS, an anionic detergent, is of much demand due to its low cost and excellent foaming properties. It finds application in household products, industry, laboratories, and pharmaceutical preparations. The high usage and disposal of this detergent is often justified putting forward its biodegradability. Sometimes, SDS is disregarded as a harmless and ecofriendly candidate. But research conducted by various scientists question the ecofriendliness of this detergent and some of them include effect of SDS on marine invertebrate embryos and larvae (Bellas *et al*., 2005), inhibition of filter feeding habits of bivalves (Ostomouv, 2003), and inhibition of mussel suspension feeding (Ostromouv & Widdows, 2006).

The present work tries to evaluate the toxic effect of SDS in fresh water habitat with reference to Grass Carp. Grass Carps are found in Kerala backwaters and are edible too. Thus the effect of detergents on Grass Carp becomes relevant.

Materials and Methods

Juveniles of Grass Carp *Ctenopharyngodon idella* (4.17 ± 0.5 cm length, 2.1 ± 0.1g wet weight) were obtained from Pumba hatchery, Alleppey district, Kerala. The fishes were held in two closed recirculating systems (200 l) for 15 days to acclimatize to laboratory

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conditions. During acclimatization period about 40% of the water in each recirculating system was replaced daily. Throughout the acclimation period and toxicity tests, fish were fed at 5% and 2.5% body weight twice a day with commercial Carp pellet feed.

The 96 h toxicity test was conducted using 65 juvenile Grass Carps. Five specimens were used as controls and the remainder divided into four lots A, B, C and D which were exposed to 5, 7, 10 and 15 mg/l of SDS (Merck) concentrations respectively. The toxicity test was undertaken in accordance with APHA (1985) with modifications. Three replicates were employed for each SDS concentration with 5 fishes in each replication in 10 l plastic containers. To avoid variations in detergent concentration, test solutions were changed every 12 hours. The biodegradation occurring within 12 hour is less than 10% of the initial concentration (Flores *et al.*, 1980). The LC_{50} value was computed using Probit Analysis Programme (Version 1.5) provided by the U.S. Environmental Protection Agency.

A total of 240 juveniles of *Ctenopharyngodon idella* were randomly selected from outdoor tanks and transferred to the laboratory at controlled temperature of 21±1°C. One third of the LC_{50} value *viz.*, 2.5 mg/l and two other nominal concentrations 2 mg/l and 3 mg/l were used for the sublethal assay. During a natural photoperiod, twenty organisms per surfactant concentration were placed in 50 l plastic containers with artificial aeration. They were exposed to sub lethal SDS concentrations of 2, 2.5 and 3 mg/l, for 30 days. A control also was maintained. Approximately one third of the LC_{50} concentration was taken as the maximum sub lethal concentration (Mishra & Bohedar, 2005). Each treatment had three replicates (4×3 = 12 sets) with 20 juveniles per container. A concentration higher than the maximum sublethal concentration *viz.*, 3 mg/l was also taken for observation to analyse the toxicity of SDS at higher concentrations.

Every 10th day of exposure till the 30th day, fish from each concentration was sacrificed. Gill tissues of the sacrificed fish were removed and subjected to histopathological analysis, and microbiological analysis. The level of antioxidant enzymes mainly superoxide dismutase and catalase was also assayed.

Gill tissues were removed and kept in 10% neutral buffered formalin. They were hydrated in isopropanol, cleared in xylene, infiltered in paraffin and sectioned at a thickness of 5 nm. Sections were stained with haematoxylin and eosin (Luna, 1968) and examined under a light microscope.

Gill filaments were excised out, homogenized in ice cold 0.25 M sucrose and potassium phosphate buffer (pH 7) and centrifuged. The supernatant were subjected to enzyme assay. A modified spectrophotometric assay of superoxide dismutase as per Kakkar *et al.* (1984) was used for superoxide dismutase (SOD) assay. Catalase activity was determined as per Claiborne (1985).

The microbial analysis of the gills was done in order to assess whether SDS exposure contributed any changes in the normal bacterial flora of the fishes. As microorganisms also contribute to tissue damage, influence of SDS on microflora of fish gains relevance. The gills of sacrificed fishes were homogenized, the supernatant after centrifugation was plated on nutrient agar plates and incubated at 37°C for 16 to 24 hours. The microbial count of SDS treated and non-treated fishes was compared. The predominant bacterial strains isolated from the fishes were identified using their morphological and biochemical characteristics as per Bergey's Manual of Systematic Bacteriology.

Results and Discussion

Details of range finding test is given in Table 1. No mortality occurred in the control groups as well as in the group exposed to

Table 1. Range finding Test

Treatments	Fishes exposed (No.)	Fishes responded (No.)	Mortality (%)
Control	15	0	_
5 mg/l SDS	15	0	_
7 mg/l SDS	15	7	53
10 mg/l SDS	15	12	80
15 mg/l SDS	15	15	100

lowest concentration of SDS during the 96 hour LC_{50} test. The 96 h LC_{50} value of SDS was computed to be 7.7 mg/l, within the upper 8.7 mg/l and lower 6.8 mg/l at 95% confidence limits as depicted in Table 2.

Table 2. LC_{50} analysis as per USEPA probit analysis software after 96 hours of exposure

Point	Exposure concentration	95% Confidence Limits	
		Lower	Upper
LC/EC 0 1.00	4.337	2.759	5.268
LC/EC 10.00	5.608	4.262	6.413
LC/EC 50.00	7.686	6.804	8.716
LC/EC 90.00	10.534	9.181	14.017
LC/EC 99.00	13.621	11.163	21.675

SDS showed profound toxic effects on Grass Carp during the study. Toxic effects of SDS on fish bioenergetics (Rocha *et al*., 2007), fertilizing ability (Rosety *et al.,* 2001) and morphohistochemical changes of gills (Rosety *et al.,* 2002) have been reported. Acute toxicity with LC_{50} values for SDS of 6.10 and 7.50 mg/l has been reported for *Sparus aurata* (Ribellas *et al*., 1995) and *Scophthalmus maximus* (Rosety *et al.,* 2002) respectively. The LC_{50} value of 7.7 mg/l of SDS on Grass Carp in the present study lies close to values described by the earlier studies mentioned above.

Progressive gill damage was noted on the 10th, 20th and 30th day of SDS exposures. The gill sections exposed to 2 mg/l SDS, after 30 days showed a generalised shortening of the gills, presence of lesions and intraepithelial oedema in Plate 2. The gills of fishes exposed to 2.5 mg/l SDS showed redness and oedema. Necrosis and complete rupture of gill tissue was found in fishes exposed to 3 mg/l SDS by the 30th day.

Histology provides a useful tool to assess the degree of damage to body organs as a result of pollution, particularly for sublethal and chronic effects. On comparison of gills of exposed fishes to control, they appeared blood red to pale brownish. A thick coat of mucus covered the entire gill filaments including primary lamellae and secondary lamellae after 10 days of exposure as supported by Cardosa *et al,* (1996). Lifting of the lamellar epithelium and lamellar fusion could be protective in that it diminishes the amount of vulnerable gill surface area (Mallat, 1985). In addition, epithelial hyperplasia and lifting of the lamellar epithelium cause a considerable increase in the diffusion distance from water to blood which may affect the normal respiratory function of the gills (Heath, 1987).

The gills of fishes exposed to SDS expressed a high catalase and SOD activity depending on the concentration and time of exposure. The antioxidant enzyme activities of fishes are depicted in Fig. 1 & 2. The SOD activity doubled in SDS treated fishes than in the normal fishes. The activity stabilized on long term exposure of 30 days showed a constant value beyond 2 mg/l SDS concentration. A five fold increase in catalase activity was observed in treated fishes than in the untreated ones. It was noticed that the

Table 3. External microflora of fish exposed to SDS (10^5 cfu/mg tissue)

Treatment case of SDS mg/l	Incubation period (days)		
	10th day	20th day	30th day
Control	164 ± 4.00	18.6 ± 6.1	57.3 ± 3
2	142 ± 2.52	65.6 ± 23	125 ± 3
2.5	156 ± 1.53	54.6 ± 22	226 ± 1
3	56 ± 1.53	36 ± 7.0	232 ± 7

(Values are average of 5 replications) * 0th day 185 ± 6

Plate 1-4. Histopathologic analysis of Grass Carp gills exposed to SDS

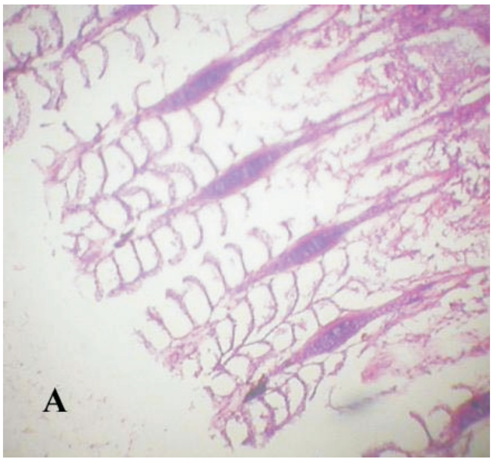

Plate 1. Control 30 days

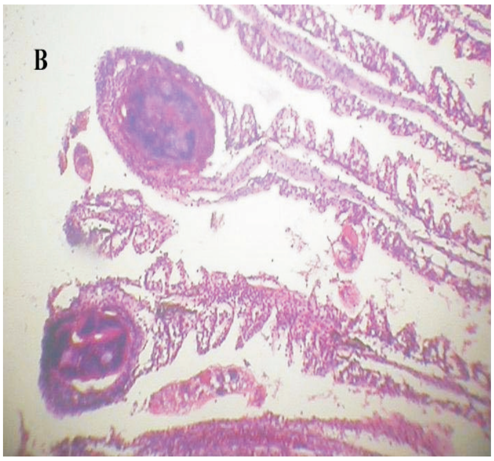

Plate 2. 2 mg/l SDS 30 days

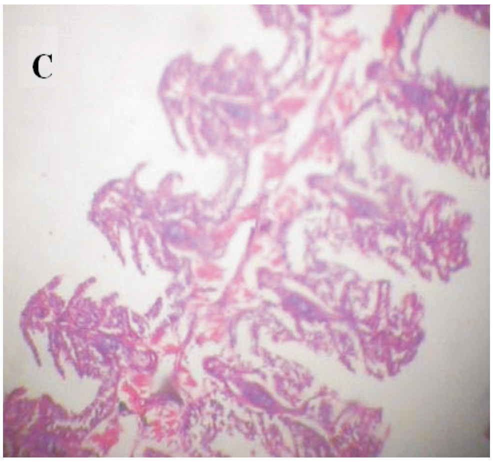

Plate 3. 2.5 mg/l SDS 30 days

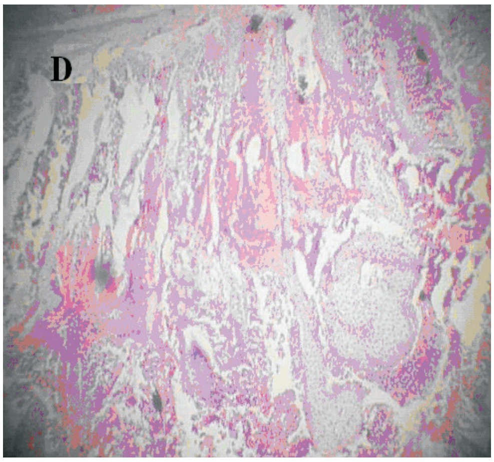

Plate 4. 3 mg/l SDS 30 days

catalase activity in fishes increased upto a SDS concentration of 2 mg/l and then decreased irrespective of increase in concentrations of SDS. This may be caused by the nonformation of H_2O_2 beyond 2 mg/l due to inhibition of metabolic process or cell necrosis as shown from histopathologic studies.

The exposure to sublethal SDS concentrations caused an increase in SOD and

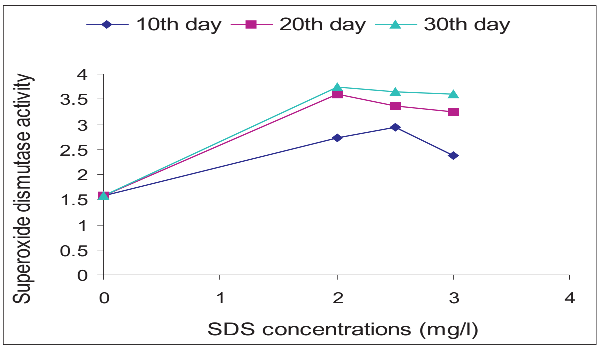

Fig. 1. Superoxide dimutase activity of gill after 10,20 and 30 days exposure to SDS

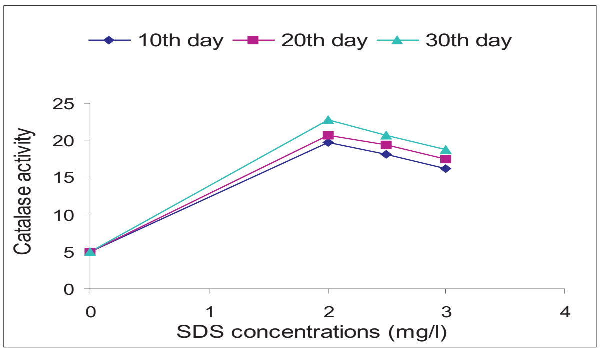

Fig. 2. Catalase activity of gills after 10, 20 and 30 days of SDS exposure

catalase levels in the exposed fishes. This increased concentrations of SOD and catalase indicate that the fish was in high oxidative stress. An induction of antioxidant enzymes like catalase, SOD and lipid peroxidation due to acute chromium toxicity in Oreo*chromis mossambicus* was observed by Kelley *et al*. (2004). The increase in antioxidant enzymes provide an additional protection against the cytotoxic effects of biologically active molecules as suggested by Paris-Palacious *et al*. (2000) and reflects an adaptive response against oxidative stress.

It was noted that SDS treated fishes showed a predisposition to microbial attack than the control fishes as shown in Table 2. The exposure to detergents cause damage to mucus layer of fishes which predispose them to microbial attack. The microbial flora of the control fishes showed a decreasing trend by the 30th day. However, the treated fishes showed an increasing microbial count. The predominant microbial flora of the control fishes included *Aeromonas hydrophila*, *Micrococcus* and *Bacillus* sp. The SDS exposure brought about a change in the microbial flora of the fish gills with the predominant presence of *Aeromonas hydrophila* and the absence of *Micrococcus* and *Bacillus* sp.

The variations in the microbial content of the fish gills suggested the role of *Aeromonas hydrophila* in tissue damage. *Aeromonas,* a normal flora of fish, also acts as an opportunistic pathogen in drastic environmental conditions. This bacterium is known for its pathogenic effects as per Kumar *et al*. (1986). The extensive necrosis noted in gill tissues, leukocyte invasion and microbial attack together suggest that SDS incites an inflammatory response in gill tissue. The extensive damage of gill tissue brought about asphyxiation and oxidation stress in fishes, which is evident from the increased level of anti-oxidant enzymes like SOD and catalase. This is in support of Abel (1976) who reported that the pathological effect of SDS is an acute inflammatory response characterized by leukocyte invasion and oedema of gill tissue. With increasing concentration, the severity of the inflammation increases.

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