Histopathological changes in testis of the freshwater fish, *Heteropneustes fossilis* (Bloch) exposed to linear alkyl benzene sulphonate (LAS)

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**Abstract:** The objective of this study was to use the freshwater food fish, *Heteropneustes fossilis*, as a model to evaluate the concentration-dependent effects of LAS on the testicular structure through short-term static bioassays. Concentration mediated histopathological lesions were observed in testes of *H. fossilis*, treated with four different concentrations of LAS for 24, 48, 72 and 96 hr. Inference drawn from the study is that the cytotoxic damage is more pronounced in fish exposed to higher concentrations of LAS for shorter durations than lower concentrations of LAS for longer durations. Gross damage of germinal epithelium, inflammatory response, intertubular vacuolations and contraction and condensation in the cells of tubules under all sets of intoxication and exposures are quite suggestive of reproductive impairment leading to delayed gonadal maturity.

**Key words:** *Heteropneustes fossilis*, Detergent, LAS, Testis, Surfactant, Leisions

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**Introduction**

India is endowed with rich fishery resources for the development of aquaculture. Its strategic location in the Indian ocean, with two monsoons per year, the tropical climate, numerous inland water bodies, coastal estuaries and lagoons are a unique gift of nature for vast potential for aquaculture. Thus, by adopting aquaculture practices even at small scale it is possible to increase the aquatic food production from the present 5 million to at least 15 million tonnes. However, the production of fish is being adversely affected due to nonsustainable activities of man that exert both, direct and indirect effects upon fish fauna. The former is expressed in the form of over fishing while the latter through altering the physico-chemical status of the aquatic environment by discharging treated, untreated industrial and domestic effluents. The polluted water destroys the suitable conditions needed for reproduction and also disrupts the metabolism of fishes leading to the large-scale mortality for a number of times.

Soaps and detergents are one of the common pollutants, which causes pollution of inland water at tremendous pace and with the advent of potent anionic surfactant chemicals e.g. LAS, there has been a spurt in both production and consumption of a variety of detergent formulations. In India total annual requirement of LAS was estimated to be about 1.5 to 2.0 lac tonnes which gained further momentum on account of their easy biodegradability. Previously detergent induced dermatological, teratogenic, biochemical and histological insults have also been documented by several workers in higher animals (Palmer et al., 1975; Mathur et al., 1987; Nomura et al., 1987; Agarwal et al., 1990; Ishii et al., 1990; Trivedi et al., 2002; Rana and Verma, 2005; Liwarska-Bizukojc et al., 2005; Mathur, 2005; Millar et al., 2005). Indiscriminate discharge of detergents through effluents have caused various ecophysiological disorders in fish fauna (Roy, 1988; Toshima et al., 1992; Mittal and Garg, 1994; Whiting et al., 1996; Kline et al., 1996; Dom et al., 1997; Trivedi et al., 2001; Rani et al., 2002; Sharma et al., 2004; Shahsavani, 2004; Bakirel et al., 2005; Saxena et al., 2005; Chukwu and Odunze, 2006). Fishes are quite susceptible to detergents and their continuous exposure even with to concentrations may reduce the resistance of fish against parasitic infections.

Moreover, exposure of fishes to detergents cause destruction in gill epithelium, impairment of chemoreceptor organs, and damage to epidermis and pharyngeal walls (Bardach et al., 1965; Brown et al., 1968). In our experiments too, we have observed impairment in gill architecture along with heavy discharge of mucus in fish exposed to LAS. As for the noxious nature of detergents, the present study investigates the breeding potential of a common food fish, *H. fossilis* by evaluation of histological architecture of testis under LAS (surfactant) stress.

**Materials and Methods**

Live specimens of *H. fossilis* were carefully hand netted from Gomti river and soon transported to the laboratory in wide mouthed large plastic containers in natural water to avoid stress and injuries. To remove external infections fish were washed 5 times in tap water then treated with 2% KMnO$_4$ solution, 0.01% formalin solution and benzalkonium chloride (1 to 4 ppm) solution for 3 hr, 15 min. and 1 hr, respectively. They were examined thoroughly for external parasites at gills and through general blood picture (GBP) for blood parasites. Uninfected and healthy fish of average weight and length (15.8 ± 1.24 g and 14.0 ± 1.34 cm) were transferred to large glass aquaria and acclimatized for 10 days prior to experiment. Commercial fish food was provided to fish and continuous artificial aeration was maintained throughout the acclimation and exposure periods.

The fish were assigned forty groups of ten specimens each and for each exposure period a series of 10 concentrations of test
chemical (LAS) were selected in logarithmic ratio and ten specimens were subjected to each of these concentrations. The mortality of fish was recorded at regular intervals. The raw data generated were fed in a Pentium Computer having the required software for the estimation of $LC_{50}$ values.

For each set of experiment ten specimens of acclimatized fish from original stock were subjected to 0.48 ml/l, 0.28 ml/l, 0.18 ml/l and 0.03 ml/l concentration ($LC_{50}$ values for 24hr, 48hr, 72 hr and 96 hr, respectively) of test chemical for different exposure periods. Similar number of fish was maintained in controls for similar duration of exposure. For both the purposes 4 g/l water load was maintained (Burrress, 1975).

Physico-chemical characteristics of water were analyzed at the onset of the experiment and at the regular intervals of 24h, using standard procedure of APHA (1995) for various parameters, such as alkalinity, pH, DO, COD, BOD and hardness (Kumar et al., 2000). At the termination of experiment both control and treated fish were dissected in Ringer's saline, testis was excised quickly, separated from the adjoining tissues and weighed for calculation of gonadosomatic index (GSI = Total testis weight / Body weight x 100).

The serial sections of 4 μm cut with the help of 820-Spencer Rotatory Microtome, were then stained in Haematoxylin-Eosin and mounted in DPX for microscopic examination under Nikon-HFX-DX-Trinocular Microscope.

**Test chemical:**

Linear alkyl benzene sulphonate (LAS) was procured from SIGMA chemicals (USA) and neutralized by adding Na$_2$CO$_3$ solution till neutral pH was obtained. Median lethal concentration ($LC_{50}$) of LAS for different exposure periods (24 hr, 48 hr, 72 hr and 96 hr) was determined by using a software, Trimmed Spearman-Karber method (Hamilton et al., 1977). The $LC_{50}$ values for different exposure periods 24, 48, 72 and 96 hrs were obtained as 0.48, 0.28, 0.18 and 0.03 ml/l respectively.

**Results and Discussion**

Tests of *H. fossilis* are paired organs found in the abdominal region and each is enclosed in a peripheral connective tissue sheath. The innermost layer of this sheath, tunica propria, projects into the lumen of testes forming the seminiferous tubules. These tubules are lined internally with tubular or seminiferous or spermatogenic epithelium which gives rise to spermatocytes. The spermatocytes are later transformed into next developmental stage of spermatids and then to spermatozoa. Masses of spermatozoa can be seen lodged in seminiferous lobules, located at the blind ends of seminiferous tubules. This lobular part can be distinguished into somatic cells and germ cells. The central portion of the testis is made up of glandular tissue consisting of large and spherical interstitial glandular cells, fibroblasts, blood and lymph vessels (Fig. 1). Testis of *H. fossilis* shows significant changes when exposed to detergent chemical- LAS for different exposure periods. Extensive cytotoxic damage, general inflammatory response and other histological abnormalities are quite prominent. Although, inter-tubular vacuoles are clearly visible in all the four sets of LAS exposure (24 to 96 hr), the extent of histological damage, as is evident by the presence of large number of both inter and intra-tubular vacuoles, was maximum after 24 hr of exposure period having 0.48 ml/l of LAS concentration. Gross condensation of spermatogenic cells, which is evident by clump formations and appearance of inflammatory lesions are also quite prominent (Fig. 2). The extent of vacuolation in tubular epithelium increases with the increase in the concentration of LAS as it is maximum after 24 hr of intoxication. Inflammatory cells are seen in the testicular tissue of every treated fish, their number increases as the concentration of LAS increases, i.e. the effect is exposure...

![Fig. 1: Section of testis of *H. fossilis* (control) showing normal histology (H and E x 400), Intertubular vacuolation (iv) and scattered tubular cells (Tc)](image-url)
Fig. 2: Section of testis of *H. fossilis* treated with LC$_{50}$ of LAS for 24 hr (H and E x 400), distortion and degeneration of tubular epithelium (Dte), extensive intertubular vacuolation (v), condensation of tubular cells (Ctc) and inflammation (In)

Fig. 3: Section of testis of *H. fossilis* treated with LC$_{50}$ of LAS for 48 hr (H and E x 400), vacuolation in intertubular membrane (In), condensation of tubular cells (Ctc), inflammation and distortion of seminiferous epithelium (Dse)
Fig. 4: Section of testis of *H. fossilis* treated with LC$_{50}$ of LAS for 72 hr (H and E x 400), inflammation (In), intertubular vacuolation (V) and condensation of spermatocytes (Ctc)

Fig. 5: Section of testis *H. fossilis* treated with LC$_{50}$ of LAS for 96 hr (H and E x 400), condensation and vacuolation of tubular cells (Ctc) and starry sky appearances (Ss)
Histopathological changes in testis of H. fossilis exposed to LAS

LAS intoxication results in distortion of the tubular epithelium and condensation of tubular cells. Exposure dependent and concentration mediated increase in the number of inflammatory cells is evident from all the four sets of intoxication. However, maximum number of inflammatory cells can be seen after 24 hr of exposure. After 48 hr of exposure (concentration 0.28 ml/l), in addition to gross vacuolation and inflammatory response, distortion of seminiferous epithelium is quite prominent (Fig. 3). Further, exposure of LAS, even with lesser concentration (0.18 ml/l for 72 hr), results in condensation of spermatocytes besides inflammation and inter-tubular vacuolation, (Fig. 4). Histopathological findings after 96 hr of exposure (Fig. 5) having 0.03 ml/l concentration of LAS are quite near to the controls but prolonged exposure, even with low dose, has resulted in further condensation of spermatocytes. Further histological damage can be visualized in the form of shrinkage of interstitial cells and vacuolation of tubular cells, which has resulted in peculiar starry sky appearance of the testicular tissue.

Gonadosomatic index (GSI) for testis of LAS treated fish shows slight decrease from that of control. Maximum difference is observed in the fish treated with 0.48 ml/l concentration of LAS for 24 hr, which is evident from the Table 1.

In tissue damage basic protective response is expressed in terms of inflammation. The purpose of this inflammatory response is to provide the site of insult, the heavy rush of cells and tissue fluids that are best able to prevent further damage of the affected tissue despite the impact of insult. In present investigations gross inflammation was found in the testicular tissue of fish treated with different concentrations of LAS. Testicular inflammation was documented as one of the common responses in both aquatic and terrestrial animals exposed to environmental toxicants (Sokal et al., 1985; Ruby et al., 1986, 1987). Short term static bioassays conducted in our laboratory clearly indicate that exposure of LAS is responsible for histo-anatomical damage of fish testis in terms of condensation of spermatogenic cells, vacuolation of tubular cells and distortion of seminiferous cells along with inflammatory lesions. Further, appearance of these symptoms even after shortest exposure of LAS for 24 hr is really alarming and is quite suggestive of the fact that fish testes are quite susceptible to this pollutant which is also evident by 29.13% decrease in GSI. Shrinkage of interstitial cells and vacuolation of tubular cells which has resulted in peculiar starry sky appearance of the testicular tissue after longer duration of exposures (72 and 96 hr) even with low doses is a strong evidence for testicular atrophy. These studies, thus, reveal that histo-anatomical damage of testis is exposure dependent and dose mediated. These findings are also in agreement with our earlier biochemical data generated by testicular tissue in response to LAS exposure (Trivedi et al., 2001).

Although, only scanty information is available regarding LAS induced testicular impairment in fishes, present findings are well comparable with histo-pathological anomalies in tests of other animals and fishes under the impact of various environmental pollutants. The degenerative changes in seminiferous tubules, enlarged interstitium and haemorrhage in intertubular area in albino rats exposed to pesticides have been reported (Dutta and Dikshith 1973; Nigam et al. 1979) and Baronia and Sahai (1993). In the present study similar changes like testicular atrophy, damage of the seminiferous tubules and spermatogenic cells, have been observed in LAS exposed fish. These changes may culminate in the partial or total arrest of spermatogenesis. Katti and Sathyasenan (1985) observed exposure dependent and concentration-mediated changes in testis of C. batrachus treated with lead. Hilderbrand et al. (1973), Sankar and Mondal (1973) and Gunn and Gould (1975) have reported similar observations in lead treated rats. Kinnberg, et al. (2000) have also documented concentration dependent effects on nonylphenol on testicular structure of the fish, Xiphophorus maculatus. Zutshi (2005) observed, the effect of fenithion on the testes of Glossogobius giuris. They observed reduction in size with spermatids and sperms in degenerating condition. Christoffersen et al. (2003), have observed effect of LAS on potential reproduction on marine calanoid copepod population. Barbieri et al. (2002) evaluated the toxicity of LAS on Mugil platanus (mullet) in relation to the temperature and salinity. Present study, thus suggests that the extent of damage of fish testis not only depends on the concentration of the toxicant but also on the time of exposure. A continuous decrease in Gonadosomatic index (GSI) with the increase of the dose of LAS shows that this surfactant chemical causes cellular damage, checks the growth and maturation of sex cells. Finally it can be concluded that exposure to LAS, can result in decreased fertility potential in male H. fossilis.

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Table 1: Gonadosomatic Index for testis of the fresh water fish, H. fossilis treated with LC50 of LAS for different exposure periods (Mean ± SD)

<table>
<thead>
<tr>
<th>Exposure period in hr</th>
<th>Gonadosomatic index</th>
<th>Percentage change in GSI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control groups</td>
<td>Treated groups</td>
</tr>
<tr>
<td></td>
<td>0.3116 ±0.0066</td>
<td>0.2208 ±0.006*</td>
</tr>
<tr>
<td></td>
<td>0.3161 ±0.0038</td>
<td>0.2576 ±0.0093*</td>
</tr>
<tr>
<td>24</td>
<td>0.308 ±0.0028</td>
<td>0.272 ±0.0035*</td>
</tr>
<tr>
<td></td>
<td>0.3132 ±0.0046</td>
<td>0.287 ±0.0041*</td>
</tr>
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Number of samples analysed: 10; * p > 0.01, 0.001

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