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# LINEAR ALKYL BENZENE SULPHONATE, A DETERGENT INDUCED TOXICITY ON THE GILL OF *PUNTIUS TICTO* FISH

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**ABSTRACT**
Detergents are common household products used for cleaning of domestic materials. The 'after wash' of the detergents are either drained into the aquatic environment such as ponds, lakes, rivers, streams, etc. or they find their way into the aquatic environment by natural sewage. Hence in the present study, the acute toxic and histopathological effects of a detergent, "Linear Alkyl Benzene Sulphonate" (LABS) on the gill of *Puntius ticto* (a teleost fish) were evaluated. For acute toxicity study, the fishes were exposed to LABS (Henko) in graded concentrations (20-28 mg/L, except 22 mg/L) for 24, 48, 72 and 96 hr duration. All 30 (100%) fishes were died in the Henko concentration of 28 mg/L at 24 hr, indicating that the acute toxicity of LABS is dose dependent. The $LC_{50}$ of Henko was found to be 25.5 mg/L. For histopathological study, the fishes were exposed to 2 mg/L Henko for one month. The histopathological examination of Henko-treated fish gill revealed severe changes in the epithelial lining of gill arch, gill rakers and gill filaments, suggesting that the LABS may induce the abnormal cellular structure in gill.
**KEYWORDS:** Gill, Linear alkyl benzene sulphonate (Henko) detergent, *Puntius ticto* (teleost fish), Toxicity.

## INTRODUCTION

A number of experiments and the use of drugs have been successfully applied in different species of fish; therefore, fish may be used as model experimental organism in biomedical research, including pharmacology and toxicology[1]. The potential for the application of research findings to both human and environmental health issues make fish species attractive and valuable alternative models in the toxicity research[2]. A variety of chemicals have been reported to cause damage as well as standard experimental toxicity in different body organs[3-4]. 'Detergents' are the parts of a large group of chemical compounds, collectively referred to as 'surface-active agents or surfactants', which all have the property, to some degree, of reducing surface and interfacial tensions, i.e., they are emulsifying agents. In other words, the detergents are classified as emulsifying and cleansing agents which frequently possess the antibacterial properties, though the term 'detergent' has become a common household name for a chemical cleansing agent. Soaps (anionic detergents) have been used as detergents for many years to emulsify the grease and to loosen the keratin, dirt and similar debris[5].

Thus, the detergents have become one of the most commonly used household products for cleaning of domestic materials in every day of life throughout the world. The 'after wash' of the detergents are either deliberately drained into the aquatic environment such as ponds, lakes, rivers, streams, etc. or they find their way into the aquatic environment by natural sewage. Linear alkyl benzene (LAB) is a family of organic compounds with the formula $C_6H_5CnH_2n+1$. They are mainly produced as intermediate in the production of surfactants, for use in detergent. Since the 1960s resulted in LAB emerging as the dominant precursor of biodegradable detergents. LAB has been subject to concern about its effect on the environment and human health. Following the exposure assessment of LAB, the environmental risk characterization for each protection target in the aquatic, terrestrial and soil compartment was determined. LAB is sulphonated to produce "Linear Alkyl Benzene Sulphonate" (LABS), a biodegradable surfactant. The LABS (an anionic detergent, belonging to ionic group of surfactants) is the most widely used surfactants in the world, primarily in laundry detergents and cleaning products. In conventional sewage treatment plants, more than 99% of LABS is removed. LABS toxicity data ($EC_{50}$) to aquatic organisms range between 1 and 10 mg per liter in short term tests. LABS is approximately equally toxic to fish and invertebrates, whereas toxicity to algae varies widely[6]. The distribution of LABS has been found in domestic waste, waste effluent and river in the Tokyo metropolitan area[7]. The concentration and distribution of LABS have also been found in the sediments of the river Mersey (Estuary, UK)[8]. These authors have also stated that the LABS is much persistent and persists in the sediments. The detergent input in the fresh water of India has also been reported to have reached a point of serious concern[9]. According to a report[10], the LABS constitutes about 88% of all the detergents used in the United States. As water bodies become the ultimate sink for the toxic chemicals used in laundries and industries, these detergents have been proved to be highly toxic to aquatic organisms, including plank tonic forms, invertebrate fauna and fish. Hence, there has been much emphasis on studying the toxicity of such detergents in fish and other aquatic organisms[9].

In view of the above facts, the present study was performed to assess the acute toxicity and histopathological effects of LABS on the gill of *Puntius ticto* (Hamilton, 1822). *P. ticto* is a kind of the "Teleost fish", popularly known as 'Ticto barb' or 'Twospot barb'. This species has also been referred to as *Barbus ticto, Cyprinus ticto* and *Systomus ticto*, among others. *P. ticto* is a freshwater and brackish subtropical fish, belonging to the minnow family (Cyprinidae). It is a native of the upper Mekong, Salwen, Irrawaddy, Meklong and upper Charo Phraya basins in the countries of Nepal, India, Pakistan, Myanmar, Bangladesh, Thailand and Sri Lanka. *P. ticto* has also been reported[11] to be found in Madhya Pradesh (India). In gill disease, the fish appears to be swimming in a great hurry, but it does not move at all. The fins flap very fast and the fish gasp for breath at the surface or lie listless at the bottom[12].

## MATERIAL AND METHODS

Healthy freshwater teleost fish, *P. ticto* were collected from the Betwa river (also known as Vetravati or Shuktimati river, originates from Kumra village in Raisen district and flows through Vidisha district of Madhya Pradesh, and finally joins as a tributary of the Yamuna river at Hamirpur, Uttar Pradesh). The fishes were acclimatized in the laboratory for a period of one week. They were fed with a mixture of oil cake and wheat bran (50:50), and used as test animals for bioassay. The college tube-well water was used for

entire experimental work. The fishes measuring 3.5 to 5.0 cm in length were used for the experiment. The experiment was conducted during winter season (in October-November when the temperature was at $21^0 \pm 2^0$C).

The stock solution of LABS (viz., 'Henko') detergent was prepared by using diluent water. The physico-chemical characteristics of water were analyzed as per the APHA 1985. The glass aquaria of 20 litres capacity were used. For acute toxicity study, a total of 240 fishes were kept in eight glass aquaria, each having 10 fishes. A series of Henko in graded concentrations (20-28 mg/L, except 22 mg/L) were prepared by adding stock solution in 10 litres of diluent water (APHA 1985). Therefore, the glass aquaria numbers 1, 2, 3, 4, 5, 6, 7 and 8 were filled with 10 litres of diluent water containing Henko concentration of 20, 21, 23, 24, 25, 26, 27 and 28 mg/L, respectively. The mortality of fingerlings of *P. ticto* after the exposure of different concentrations of Henko detergent was recorded for 24, 48, 72 and 96 hr (Table 1). The dead fishes were removed from the test aquaria. For histopathological study, 10 fishes were kept in a glass aquarium filled with 10 litres of fresh water containing 2 mg of Henko per litre of water. The fishes were exposed in this concentration for a period of one month. Thereafter, the gills of fishes were removed and preserved. The histopathological study of gill tissues was conducted as per the standard described method, and the gill tissues were examined under the light microscope.

## RESULT AND DISCUSSION

**Acute toxicity:** The results of the acute toxic effect of LABS have been shown in Table 1. All 30 (100%) fishes were died in the Henko concentration of 28 mg/L at 24 hr. However, the overall mortality percentages (from 24-96 hr) of fishes were found to be 80, 80, 40, 40, 40 and 23.3% in the Henko concentrations of 27, 26, 25, 24, 23, 21, and 20 mg/L, respectively. The median lethal concentration ($LC_{50}$) of Henko has been calculated as 25.5 mg/L because up to 48 hr (which is a standard duration for drug exposure in acute toxicity and $LC_{50}/LD_{50}$), 12 and 18 fishes were died in the Henko concentrations of 25 and 26 mg/L, respectively; hence, half (50%), i.e., 15 out of 30 fishes may die in the Henko concentration of 25.5 mg/L. The results indicated that as the dose (concentration) of LABS increased, the mortality percentage of *P. ticto* fingerlings also increased, suggesting that the acute toxicity of LABS is dose dependent. The acute toxicity of LABS and similar other chemicals have also been reported earlier[6,9].

**Histopathological study**: Under light microscopy, the fishes (normal) not treated with LABS showed normal anatomical and surface features of gill arch, filaments and lamellae. The gill arch had two rows of gill filaments and a row of gill rakers; the former directed towards the opercular opening and the latter towards buccopharyngeal chamber. Two rows of gill filaments were directed posterio-ventrally from each arch arranged in a manner that resembled like a comb. The tips of gill filament formed a gill certain forming sieves for the passage of water passing from buccal chamber to opercular chamber. After one month exposure of LABS detergent, severe histopathological changes were observed in the epithelial lining of gill arch, gill rakers and gill filaments of fishes. Fusion of gill lamellae was observed at the tip of gill filament with accumulation of blood cells in capillary and infiltration of blood cells in sub-epithelial space. The mucous cells in gill rakers and lamellae were also seen. The secondary lamellae of the basal region of filament were also found fused with each other leaving part free, indicating proliferation (epithelial lifting) of inter-lamellar epithelia within the lamellae. In gill rakers, large empty lymphatic spaces were formed around the central row of pilaster cells.

The histopathological findings of the present study may be correlated with the observations of many researchers. Similar histopathological changes, including lifting of lamellar epithelium and formation of empty space in gill have been seen earlier by some investigators[13-15] in fish exposed to some insecticides and chemical substances. The effects of three chemical compounds, i.e., organochlorine pesticides (viz., BHC, lindane and endosulfan) on the gill structure of *P. ticto* (a freshwater teleost fish) were investigated. Histopathological examination revealed more or less similar structural and functional changes in the gills after 15 days' exposure of pesticides. Exposure to BHC (0.17 ppm) was followed by an inflammatory reaction and complete dystrophy of the lamellar structure of the gills. Lindane (0.19 ppm)-treated fish showed disruption of the epithelial covering of the gills and excessive haemorrhage in the blood vessels; and the endosulfan (0.20 ppm) exposed fish revealed shrank and thinner gill lamellae[16]. Similarly, the *P. ticto* exposed to lethal concentrations (25% and 22% for 24 hr and 96 hr of $LC_{50,}$ respectively) of distillery effluent, showed pronounced histopathological alterations in the gills, such as cloudy swelling in pillar cells. Fusion of secondary lamellae and haemorrhage at some places were also observed on the 24 hr exposure. Fusion and cytoplasmic degeneration were also observed in the secondary gill lamellae along with cell necrosis. Hypertrophy of epithelial cells with pyknotic nuclei have been noticed in pillar cells on the 96 hr exposure[17].

## CONCLUSION

From the results of the present study, it appears that the LABS detergent has acute toxic and severe histopathological effects on the gill of *P. ticto* fish. Hence, the drainage of the 'after wash' of this detergent besides other chemicals into the aquatic environment (e.g., ponds, lakes, rivers, streams, dams) should be strictly prohibited. Fish may be the model organism to detect the toxicity of different chemicals drained or contaminated in the aquatic environment.

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**Table 1: Mortality in *P. ticto* fingerlings during acute toxicity of LABS (Henko) detergent**

| No. of fish in a glass aquarium | Concentration of Henko (mg/L) | Mortality rate (total fishes died and mortality %) | | | | |
|---|---|---|---|---|---|---|
| | | At 24 hr | At 48 hr | At 72 hr | At 96 hr | Overall (24-96 hr) death & mortality % |
| 30 | 20 | Nil (0%) | Nil (0%) | Nil (0%) | Nil (0%) | Nil (0%) |
| 30 | 21 | Nil (0%) | 6 (20%) | Nil (0%) | 1 (3.3%) | 7 (23.3%) |
| 30 | 23 | 12 (40%) | Nil (0%) | Nil (0%) | Nil (0%) | 12 (40%) |
| 30 | 24 | 12 (40%) | Nil (0%) | Nil (0%) | Nil (0%) | 12 (40%) |
| 30 | 25 | 12 (40%) | Nil (0%) | Nil (0%) | Nil (0%) | 12 (40%) |
| 30 | 26 | 18 (60%) | 1 (3.3%) | 1 (3.3%) | 4 (13.4%) | 24 (80%) |
| 30 | 27 | 24 (80%) | Nil (0%) | Nil (0%) | Nil (0%) | 24 (80%) |
| 30 | 28 | 30 (100%) | Nil (0%) | Nil (0%) | Nil (0%) | 30 (100%) |

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