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Histopathological effects of detergent, Linear Alkyl benzene sulphonate on hepatopancreas of freshwater prawn, *Macrobrachium lamarrei* (Crustacea-Decapoda)

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Abstract
Freshwater prawns, *Macrobrachium lamarrei* (Crustacea-Decapoda) were subjected to static subacute exposure (0.755 mg/l) of detergent, Linear Alkyl Benzene Sulphonate (LAS) (25% of 96h LC_{50}) for 10,20 & 30 days. Hepatopancreas showed pronounced changes like vacuolization, hypertrophy, hyperplasia and degenerative changes leading to almost complete loss of histoarchitecture after 30 days exposure. Peculiar cell shedding in tubules was noticed after 10 day exposure while heavy increase in wandering cell population was recorded after 20 day exposure. R&B cells were found most effected throughout the hepatopancreas. Histopathological effects were found duration dependent. Underlying mechanism of detergent toxicity and role of histopathology as biomarker has been discussed.

Keywords: Detergent toxicity, LAS, histopathology, hepatopancreas, freshwater prawn

Introduction
With increasing awareness towards cleanliness, industrial as well as household use of detergent has increased many folds. Out of all detergents, Linear Alkyl Benzene Sulphonate (LAS) occupies a major share in market throughout globe. LAS is an anionic detergent enters in aquatic ecosystem and adversely affects to flora and fauna. Initially LAS were considered as biodegradable but now it is proved toxicants to aquatic fauna by affecting their histology, physiology and biochemistry. Toxic effects of detergents have been worked out mainly on fishes (Bardach *et al*., 1965, Abel 1974, Mishra *et al.*, 1985, Trivedi *et al*., 2001, Cambell & Tapale 2011, Eknath 2013, Binduleuhe 2018, Day *et al*., 2019, Han & Jung 2021, Kolarova *et al.*, 2021) [3, 1, 18, 28, 7, 4, 6, 9, 13]. Freshwater crustaceans, despite being important members of aquatic food chain, having high economic value and are potential animal for freshwater aquaculture have been documented less (Sharma & Shukla 1990, Sharma & Shukla 2006, Shukla *et al*., 2013) [23, 22]. Present work aims to evaluate histopathological effects of LAS on hepatopancreas of freshwater prawn, *Macrobrachium lamarrei* (Crustacea-Decapoda) which has immense economical, nutritive and medicinal value as well as can serve as a better bioindicator of worsening states of aquatic bodies.

Material and Methods
Freshwater prawns, *Macrobrachium lamarrei* (Crustacea-Decapoda) were collected from river Gomti (U.P.), Lucknow, India with the help of local fisherman & brought to laboratory & maintained in glass aquaria (Sharma & Shukla 1990, Verma *et al*., 2010) [23, 30]. Animals were acclimatized to the laboratory conditions for 5-7 days prior to experimentation. Stock solution of detergent, LAS was prepared and adjusted to pH-7.0 with Sodium Carbonate (Lal *et al.* 1983) [15]. Physicochemical characters of diluent water were as:- pH- 7.3+_0.2, Temp. 26+_20 C, D.O= 7.0+_0.1 mg/lit, Total hardness -268+_2.5 mg/lit.
For experiment two aquaria containing 20 lit water were used, in experimental aquaria 0.755 mg/L (25% of 96 hr LC_{50}) detergent was dissolved while another containing water only served as control. 20 healthy average sized, intermoult prawns (L=4.8+_0.55 cm, W= 1.07+_0.26gm) were carefully transferred to each aquaria. Air was continuously supplied with air pump while food was given on alternate day with change of experiments solution. Hepatopancreas from control experimental groups were dissected out after 10, 20 & 30 day exposure, fixed in alcoholic Bouin's fluid for 24 hrs.

Paraffin blocks were prepared & sections of 5-6 u were cut on rotatory microtome and stained with Harris Haematoxylin and Eosin. Stained slides were studied and photographed by Olympus Trinocular Microscopic, comparing with controls. Experiment was replicated thrice.

Results and Discussion

Linear Alkyl benzene sulphonate showed pronounced effects on histological architecture of hepatopancreas of *M. lamarrei* comparing with controls (Plate-1, Figs 1 & 2). After 10 day exposure (Figs 3 & 4) hepatopancreatic tubules showed vacuolization and deposition of granular material mainly inside B&R cells. Heavy cell shedding was observed and lumen of tubules were found filled with lysed cells & nuclei. Luminal brush border of tubules was found damaged at places. Heavy hepatocytes infiltration in tubules as well as intertubular spaces were noticed at this stage.

After 20 days exposure (Figs 5 & 6) the lumen of most of tubules were found filled with destroyed cells, pyknotic nuclei & granular material. Tubular epithelium becomes very thin due heavy destruction. Increased lumen and almost complete loss of luminal brush border was noticed in most of the tubules. Granulomatous depositions in intertubular spaces were common. Migration of fibroblast & phagocytes type cells was more pronounced in intertubular space at this stage.

After 30 days exposure (Figs 7 & 8) almost complete loss of histoarchitecture of hepatopancreatic tubules was observed due to severe necrotic, lytic and degenerative changes. Almost 60% of the tubules were found damaged and remaining tubules were highly thin leaving tunica propria with dialates lumen filled with granular material. Karyolysis and Karyorrhexis were common features in the tissue debris of lumen. Presence of broken cells, phagocytes and fibroblast like cells was more pronounced in intertubular connective tissues. Major affected cells were B and R cells throughout hepatopancreas. Severity of histopathological changes were duration dependent.

Crustacean hepatopancreas is a pivotal organ of body metabolism performing various functions like secretion of enzymes, digestion, absorption, storage of reserve food, nutrients and excretion etc. It is formed by extension of caecae at function of foregut and midgut, branched in racemose manner revealed that hepatopancreatic tubules are formed mainly of 4 types of cells *viz*.- B, R, F & E cells. The structure of hepatopancreas of *M. lamarrei* (Stainer *et al*., 1968, Gibson 1979, Vogt 1984, Shukla & Sharma 1992, Correa *et al*., 2002, Sausa & Petriella 2007) [27, 8, 24, 5, 26] is almost similar in Palaemon (Pathwardhan, 1937) [34]. The changes observed in present study like vacuolization, granuloma in B and R cells, necrosis of tunica propria and damage of Luminal brush border in hepatopancreatic tubules of prawns in the present study are in accordance with the earlier studies (Kutlu *et al*., 2002, Katlu *et al*., 2005, Yang *et, al*. 2007) [14, 11, 33] on crustaceans and Fishes observed in case of detergent and heavy metal toxicity as well as in the infections due to virus and other microorganism (Vogt and Quinitio 1994) [31].

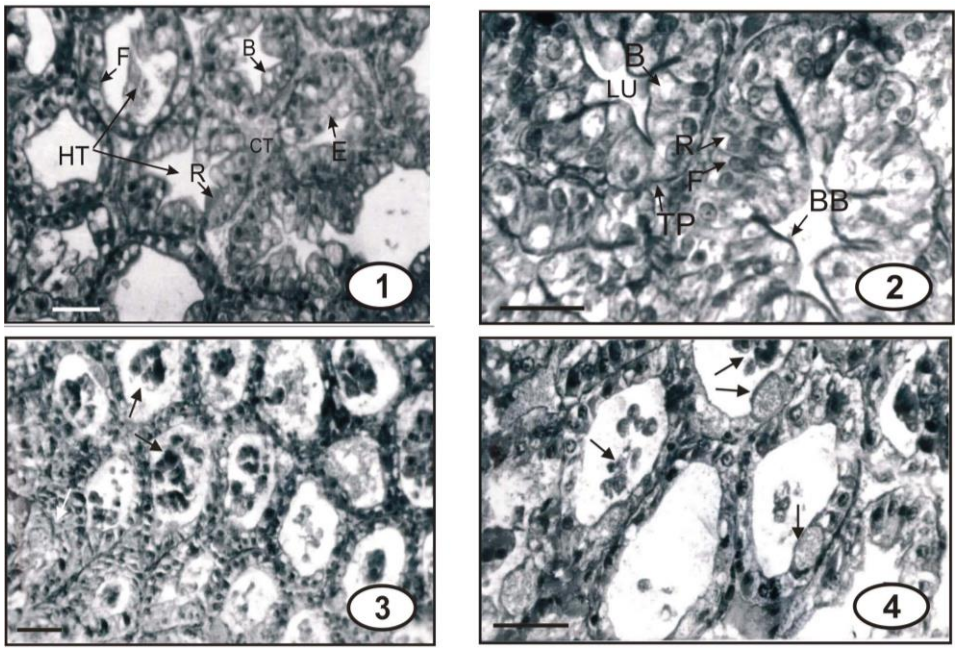
Heavy cells shedding in the tubular luman may be due toxicant induced stress as reported in other studies (Lozzi 1971, Gibson and Barker 1979) [17, 8] as well as in acute starvation and stressful conditions. Heavy infiltration of Haemocyte in the intertubular space may be an immunological response to damage of the cells due to toxicants (Yang *et al*., 2007, Lodhi and Shukla 2020) [33, 16].

Granular depositions in the cells as observed in the present study may be a protective device as reports in other crustaceans in case of heavy metal toxicity like Hg and Cd (Anderson and Boatrup 2007, Kaoud and Ahmad 2013) [10].

Complete loss of cellular architecture of hepatopancreas as observed in present study may be due to direct cytotoxic action of toxicant or due to induced secondary physiological or immunological response in the prawns as observed in present study. Major affected cells B and R as observed in present study are also in accordance with the earlier reportings (Yang *et al*., 2007, Rosales *et al*., 2010, Nagueira *et al*., 2013) [33, 21].

B and R cells are secretory, resorptive and storage cells and major damage to them hampers proper functioning of hepatopancreas, a central organ involved in crustacean metabolism.

Histological parameters can serve as a better biomarker giving holistic approach rather than isolated physiological and biochemical parameters in freshwater prawn *M. lamarrei* (Crustacea-decapoda) which is an animal of economic importance as well as better bio-indicator for aquatic contamination.



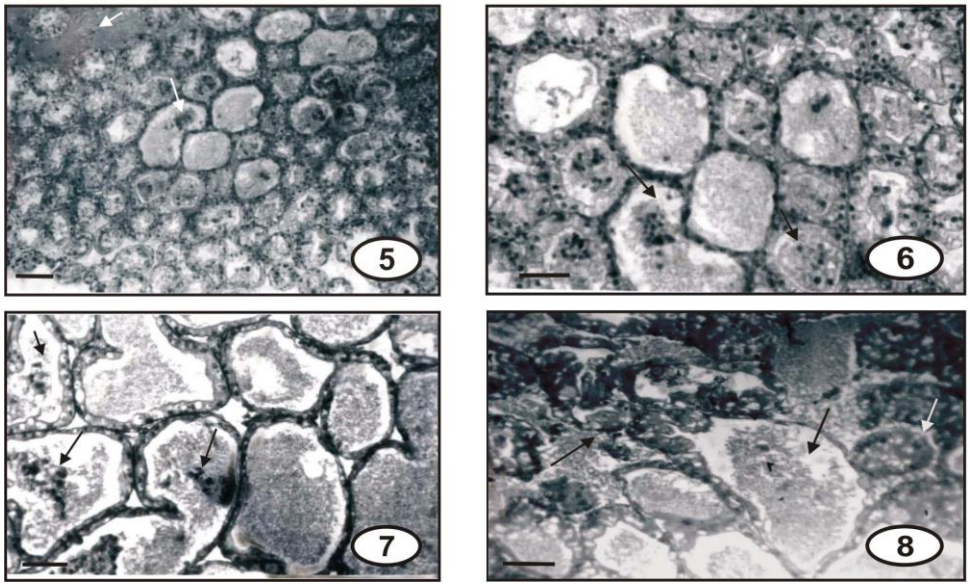


Plate1: Photomicrograph of T.S of hepatopancreas of *M. lamarrei*
1&2- Control; Fig 3&4- After 10 day exposure of LAS
5&6- After 20 day exposure of LAS, 7&8- After 30 day exposure of LAS. (Scale bar 1, 2, 3, 4, 6, 7, 8= 50µm; 5= 100µm)
(B= B-cell, E= Embryonic cell, F= F-cell, HT= Hepatopancreatic tubules, R= R-cell, BB= Brush border, TP= Tunica Propia, CT= Connective tissue)

Conclusion

Present study indicates that detergent, linear alkyl benzene sulphonate is toxic to the hepatopancreas of freshwater prawn *M. lamarrei* (Crustacea-Decapoda). Histopathological parameters of hepatopancreas may serve as better biomarker for the detergent toxicity and M. lamarrei can serve as better bioindicator of worsening state of rivers and other aquatic bodies.

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