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Efficacy of detergent on hepatocyte morphological changes in fish, *Tilapia Mossambica*

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Abstract
An investigation was carried out to study the efficacy of synthetic detergent on hepatocyte changes in *Tilapia mossambica.* In these experiments study we used detergent expose to 60 numbers of fish individuals for five month durations. The Study was divided into two (Control and Experiment) groups containing 30 individuals in each group. The control group, the fishes were exposed to proven pure water from our laboratory; Department of Zoology, the experimental group fishes were exposed to 1ppm dilution of commercial biodegradable detergents. The control and experiment study group was replicated to confirm the results. The hepatocyte observed in the control group shows the homogeneous cytoplasm and central nucleus. The experiment fish hepatocytes shows with the nuclei displaced to the periphery, some of the vacuolized nuclei or at the beginning of vacuolization process. The present results suggest the detergents were affected the fish morphology and physiological process. Future, we investigate the biochemical and metabolically aspect of these fishes of *T. mossambica*.

Keywords: Synthetic detergents, *Tilapia mossambica*, hepatocytes

1. Introduction

Environmental pollution is becoming a problem of greater concern because it has damaged the quality of water [1]. The aquatic biotas continue to suffer great chemical stress because of anthropogenic influence. Water pollution is commonly associated with the discharge of domestic, industrial or agricultural effluents. These practices generating a large quantity of residues and un-loading it on water bodies are a cheap and efficient way to get rid of most of these contaminants [2-3]. There are hundreds of pollutants that affect the aquatic environment and their effects cause great concern. The main types of pollutants are metals, organic compounds (such as pesticides, PCBs, aliphatic hydrocarbon components, solvents, surfactantes, petroleum hydrocarbonets, organometallic compounds, gases, such as chlorine, anions, such as cyanets and sulphets, acids and alkaloids. This number grows annually, considering that new compounds and new formulations are synthesized [1, 3]. This concern was corroborated by Rodrigues group; they have reported that if on the one hand the human population prospers; on the other it raises environmental risks due to production of new chemical elements that accompany this growth [4].

They have reported that human activities are changing the biosphere and causing a crisis in biodiversity, because new substances are polluting water and causing environmental catastrophes in aquatic systems. This is a very important problem and basic research should be carried out to generate useful information for future predictions so that protection strategies for these environments can be elaborated [5].

The synthetic detergents are the most used substances in modern civilization, being used to prepare shampoos, domestic cleaning products and toothpaste and are now one of the largest sources of water pollution [6]. Among the environmental contaminants, synthetic detergents released in aquatic ecosystems, with potential to affect the fauna and flora are extremely important. Eleven fish and aquatic invertebrate species exposed to mixtures of alkyl benzene Sulfonate, the active agent of detergent, in various environmental conditions and during different development stages, showed varied responses [7].

They have reported that the liver has vital functions for basic metabolism and is the organ that accounts for accumulation, biotransformation and excretion of contaminants in fish [8]. Many xenobiotics and metals accumulate in the liver, so that its cells are exposed to a high level of chemical agents that may be present in the environment or in other organs of the fish [1]. Due to its function in metabolism and its sensitivity, the liver has stood out in toxicological studies related to contamination of various fish species by organic and inorganic chemical agents. Liver histopathology in fish is a monitoring tool that indicates the effects of stress agents in fish populations and is proposed as one of the most reliable biomarkers for use in studies of environmental impact on water animals [8]. The present study focused to investigate morphology of hepatocytes in the *T. mossambica* by the effect of detergents toxicity.

2. Methodology

2.1 Specimens

The fish species of *Tilapia mossambica* were collected from Poondy Reservior, at Thiruvallur District, Tamil Nadu, India. The study was done during the period of March 2016 to July 2016.

2.2 Experimentation

The Fish were divided into two groups containing 30 individuals each. The first, considered the control group, was exposed to proven pure water from our laboratory; Department of Zoology the second group was exposed to 1ppm dilution of commercial biodegradable detergents Linear Alkylbenzene Sulphonate (LAS), Sigma. Experiments were replicated to three times with same duration for confirm the results.

2.3 Preparations for Histological Analysis

After the five month duration of experiment the fishes sacrificed. Before sacrificed the animals were anesthetized with a benzocaine solution (0.1g in 1mL ethylic alcohol for every 100 ml of deionized water). After anesthesia, in each sampling six individuals were sacrificed per group. From each individual, liver fragments were removed and fixed in aqueous Bouin solution for 48 hours at 4 C. The material was then buffered in sodium phosphate solution pH = 7.4, dehydrated at increasing alcohol concentrations, embedded in Leica historesin and sectioned with a Leica RM2245 microtome. 2.4

2.4 Hepatocyte Morphology Analysis

For this analysis five 6µm sections were obtained from each individual under study, which were submitted to the Hematoxylin and Eosine reaction [15]. This material was photographed with 40X objective lens on the Leica DM2000 microscope and the main morphological aspects of the hepatocytes were analyzed, such as nucleus position, presence of steatosis and nuclear and cytoplasmic vacuolization. In addition, the nuclei and the hepatocyte cytoplasm areas were analyzed. For such, 50 cells chosen randomly from each section obtained from the specimens under analysis were quantified, summing up to 250 cells per individual in each treatment. The measurements were made using Image J version 1.46 segments were traced around the nuclei and cytoplasm to obtain the respective areas (Figure 1 (A). Only cells with clear nuclear and cytoplasmic limitations were analyzed, so there were no errors during quantification. From the data obtained the arithmetic mean was calculated for each one of the parameters analyzed in each individual of the three treatments and these means were compared statistically.

2.5 Statistical Analysis

The data obtained were analyzed using BioEstat 5.0. To identify variations among the results, the Shapiro-Wilk normality test was applied and later the ANOVA/Tukey or Kruskal-Wallis/Dunn test of variance was performed, depending whether the data in question were parametric or non-Parametric.

3. Results

3.1 Hepatocyte Morphology

The hepatocytes observed in the control group had homogeneous cytoplasm and central nucleus. The treatment with detergent dilution showed hepatocytes with nuclei displaced to the periphery, some with vacuolized nuclei or at the beginning of vacuolization process, for both control and experimental group hepatocyte morphology results shows (Table 1.) Only the group exposed to detergent for five months showed rounded vesicles, characteristics of lipids, in this case indicating the pathology called moderate steatosis (Figure 1 (A), (B), (C), and (D).

Regarding the analysis of nucleus area and cytoplasm area, decrease in the nucleus area was observed only in the treatment with detergent after one month of experiment with $p<0.01$ for the ANOVA/Tukey test, compared to the control. The data obtained for the cytoplasm showed decreased area in the detergent group in the two experimental periods, in the first month with $p<0.01$ for the ANOVA/Tukey test and in the fifth with $p<0.05$ for the Kruskal-Wallis/Dunn test (Table 1).

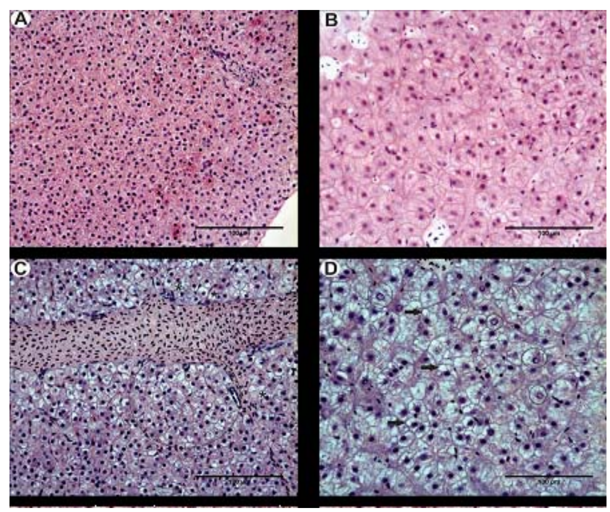

Fig 1: (A) and (B) Morphology of normal liver in *T. mossambica* note the homogeneity of the cytoplasm including the provision of the central nucleus, (C) Liver of *T. mossambica* after exposure to detergent, note the cytoplasmic degeneration (highlighted area) and cells with nuclei displaced to the periphery (*); (D) (A) Liver ------------ after exposure to the detergent note vacuolation of the cytoplasm present throughout the tissue and the presence of lipid deposits (arrow) and vacuolated nuclei (highlighted area), and-Liver of *T. mossambica* after exposure to water from the urban lake, note the cytoplasmic degeneration (highlighted area).

Table 1. Changes in the morphology of hepatocytes for the species *Tilapia mossambica*

Hepatocyte view	Control		Detergent	
	1st Month	5th Month	1st Month	5th Month
Cytoplasm Vacuolization	0	0	++	+++
Vacuolized Nuclei or the Start of Vacuolization	0	0	++	++
Nuclei Displaced to the Periphery	+	+	++	+++

0 = absent; + = uncommon; ++ = common; +++ = very common;

4. Discussion
Liver histopathology has been used as an environmental stress indicator because it is usually the endpoint of pollutants and its alterations depend on the contaminant´s nature, its concentration and exposure time [8]. The hepatic lesions were classified in groups and ranked according to their importance as an indicator of environmental contamination; non-neoplastic lesions, such as nuclear polymorphism, are considered initial reactions of exposure to toxic agents [8]. Hepatocellular necrosis is considered a response to severely contaminated environments, especially by metals [9-10]. In *Tilapia mossambica* species, two stages of liver degeneration were identified after exposure to diflubenzuron (DFB) insecticide and stage one was characterized by the presence of melanomacrophages, cell hypertrophy and nuclear and hepatocyte nuclei displacement to the periphery; the second stage presented nuclear degeneration, bile stagnation and pyknotic nuclei.

The present study demonstrated alterations such as cell hypertrophy, nuclei dislocated to the periphery, melanomacrophage aggregates reported by the authors quoted above. When compared with the study by [11] even though the modifications found appeared similar, it was not possible to fit these data in either of their two stages, since in both species characteristics of the two stages were found simultaneously [11]. The results of the present study may be fitted more clearly in the identification and the anomalies found can be inserted in the non-neoplastic group [8]. Although the water analysis showed the presence of metals in both the contaminated treatments at higher concentrations than the control, necrotic hepatocytes were not found. This showed that these pollutants were at weaker dilutions, but that their presence, together with other substances, damaged the hepatic tissue.

They have reported tilapia (*O. niloticus*) exposed to copper and observed increase in hepatocyte nuclear size, and in *Brachydanio rerio*, who also tested exposure to copper, suggesting that this alteration might indicate increase in metabolic activity resulting from detoxification [8, 12, 13].

In the present study decrease was only found for the nucleus area, but these could not be classified as pyknotic because their shape was still defined and there was no condensing of the genetic material. This decrease in area may indicate the beginning of the process to form these inactive nuclei, which result in cell death. The same studies quoted above reported that generally increase in nuclear size is accompanied by cytoplasm dilation, both indicating high metallic activity. In the present study this type of alteration was identified but only accompanied by smaller nuclei, indicating that the cells may be in high detoxification activity, transitioning to the cell death process [1].

There are few studies on the effects of detergent in fish liver; the results showed that *Carassius auratus* exposed to detergent can bioaccumulate surfactants in the liver. They have demonstrated that *Lates calcarifer* exposed to LAS presented hepatocyte vacuolization, directly linked to delayed growth [14]. The results presented in the present study showed much more serious alterations than those observed and due to the fact that the effects of detergent and other contaminants, especially the metals, were enhanced when mixed as described [1, 15].

5. Conclusion
This study showed that analysis of the effects of surfactants on aquatic fauna should be taken into consideration, but studies should be focused on the action of these pollutants together with other contaminants, as it occurs in nature. It is also important that more precise analyses, such as quantification of cytoplasm and nucleus area, should be adopted in environmental studies.

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