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ANIONIC DETERGENT INDUCED DNA DAMAGE IN FISH *CHANNA PUNCTATUS* (BLOCH,1793)

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ABSTRACT
The aquatic ecosystem is under the constant stress of pollution since most chemicals ultimately reach waterways directly through partially filtered or untreated sewage and adversely affect flora and fauna, particularly fish. Among all pollutants, anionic detergents exert major impact on account of its huge production and consumption worldwide. After absorption through gills or skin in fishes, *in vivo* metabolism of detergents leads to production Reactive Oxygen Species (ROS). The ROS reacts with biomolecules, DNA in particular, and oxidatively damage them, therefore, contributes to genotoxicity. To evaluate, detergent induced genotoxicity, the fish, *Channa punctatus* were exposed in three different groups, *viz.* a control group (T1) which was without any test chemical and two different treatment groups, T2, exposed to $1/20^{th}$ of 96h LC_{50} and T3 exposed to $1/10^{th}$ of 96h LC_{50} concentrations of detergent. Blood samples were collected after interval of each 24h up to 96h exposure periods. The treated group T2 showed a significant ($p<0.05$) changes in induction of MN frequency both after 72h and 96h of exposure periods. The group T3 also showed a significant ($p<0.05$) change in induction of MN frequency after 24h, 48h, 72h and 96h exposure periods in comparison to control group T1. The findings of the study verify the detergent induced genotoxicity in fish *Channa punctatus* and proved that DNA damage in terms of micronuclei induction could be used as efficient biomarkers in response to detergent pollution load. Thus, present studies have ample scope in early monitoring of freshwater bodies apart from regulating fish population in stressed aquatic ecosystem.

KEY WORDS: Anionic detergent, *Channa punctatus*, Micronuclei, DNA damage.

INTRODUCTION

Many countries are facing a major problem of water pollution since most chemicals ultimately reach waterways directly through partially filtered or untreated sewage. One of the main sources of chemical pollutants is everyday used detergents. Their large scale usage *viz.* solubilization, emulsification, sterilization and laundry and cleansing (L & C) operations, and disposal of untreated effluents grossly contaminate our pristine aquatic habitats and are thus constantly affecting negatively the environment (http://www.cleancult.com/blog/pollutants-in-laundry-detergent). Further, this discharge after accumulation induces damages to such vital organs like liver, kidney gills, skin, heart and brain of fishes *(Ali et al*., 2014; Misra and Trivedi, 2017). Among all pollutants, anionic detergents in particular, show major impact, since it is estimated that they are consumed at the rate of 2 billion kg per year, worldwide (Sobrino-Figueroa, 2018). The Global market of surfactant chemical is expected to reach $44.9 Billion by 2022 from $36 Billion in 2017 at a compound annual growth rate (CAGR) of 4.5% from 2017 to 2022 (https://www.reportlinker. com/ p05273318).

In vivo, detergents enter into the cell and results in greater production of Reactive Oxygen Species (ROS) which ultimate cause genotoxicity and cytotoxicity (Shukla and Trivedi, 2017). All organisms have the inbuilt ability to synthesize and control specific enzymatic systems which can be used for removal of ROS (Kumari et al., 2014). When there is an imbalance in the production of ROS and antioxidant enzyme, this stage is called oxidative stress (Olsvik et al., 2005). When above mentioned condition exists in cell for longer duration it ultimate cause DNA damage (Ratn et al., 2017). Previous studies establish that overproduction ROS can induce DNA damage by directly binding to DNA molecules, contributing to genotoxicity (Patlolla et al., 2008; Kumar et al., 2013;Awasthi et al., 2018) Toxicological relevance of micronuclei assay is convincing enough as these are formed only after DNA damage as is evident from the processes of their formation, ie., condensation of acentric chromosomal fragments and whole chromosomes, that are not included in the main nuclei during anaphase, inclusion (Yadav and Trivedi, 2009;Luzhna et al., 2013). Micronucleus test is much favoured in fish because unlike mammals, erythrocytes are nucleated and thus facilitate easy scoring (Ratn et al., 2018;Farah et al., 2006). The formation of micronuclei against detergent toxicity, thus, can be regarded as an efficient biomarker of DNA damage. Further, for biomonitoring programmes fishes are considered as ideal indictors due to their sensitivity and responses against pollutants. Moreover, they belong to higher trophic level and an important part of food web thus, any toxicant can easily accumulate in them through biomagnifications. This is an indirect threat for humans. Thus, in present studies, an attempt has been made to find out DNA damage induced by anionic detergent by using micronuclei test in fish *Channa punctatus*.

MATERIALS AND METHODS

Test animal

In the present study, the fresh water air-breathing fish, *Channa punctatus*, has been selected as test animal. For the experiment, healthy and live specimens (21 ±3.0 g; 13.5 ±1.5 cm) of fish were procured from lentic habitats of Lucknow, India. After washing with tap water, the specimen fish were given prophylactic treatment by bathing them in 2mg/l solution of $KMnO_4$ for one hour followed by 0.05% formalin for 15 minutes, respectively, to remove dermal infections. They were then acclimatized under laboratory condition (Temperature 14-22°C, dissolved oxygen 6.62-6.76 mg/l, alkalinity 62-68 mg/l) for ten days in glass aquaria (90x40x40cm) following the standard procedure (APHA *et al*., 2012). The specimens were fed with minced goat liver, dried fresh water prawns and *Chironomous* larvae at sunrise and sunset and the aquaria water was replaced on daily basis. All experimental sets were conducted in triplicate with their respective control having a water load of 4g/l of fish body weight (Burress, 1975) in the semi-static system. The chemical sodium docecyl-benzene sulfonate (SDBS) or LAS (CAS No. 25155-30-0) was procured from Sigma-Aldrich, USA.

Sub lethal concentration of LAS (LC_{50})

The LC_{50} 96h value of anionic surfactant, Linear alkyl benzene sulphonate (LAS), for fish *Channa punctatus* is considered for the study from the previous study of Shukla and Trivedi (2017) and considered as 34.40 mg/l, with 95% upper and lower confidence limits of 30.40 mg/l and 38.28 mg/l, respectively.

In Vivo exposure experiment

The fish were exposed to three different groups, *viz*. a control group (T1) which was without any test chemical and two different treatment groups, T2, exposed to $1/20^{th}$ of 96h LC_{50} and T3 exposed to $1/10^{th}$ of 96h LC_{50} concentrations of anionic detergents. The exposure was continued for 96 h, and blood samples were collected at the intervals of 24, 48, 72 and 96 h post-exposure periods. The physicochemical properties of test water, *viz.* temperature, pH, conductivity, dissolved oxygen, chloride, total hardness and total alkalinity, were analysed using standard methods (APHA *et al*., 2012).

Micronuclei test

Fish blood samples were smeared on clean and dried microscopic slides and fixed with absolute methanol for 5 min and left to air dry at 25 °C for 1 h. Fixed slides were stained with May-Grunwald's solution 1 and 2 for 3 and 5 min, respectively followed by 5% Giemsa staining for 30 min. Dried slides were mounted with DPX and observed under oil immersion microscope (Nikon Corporation K 12432) using 40/100X objective lenses. Micronuclei were scored by following the protocol adopted of Fenech et al., (2011). A minimum 1000 erythrocytes for each specimen were examined. Micronuclei frequency was calculated as follows:

$$\%\ \text{frequency of MN} = \frac{\text{Number of cells containing micronuclei}}{\text{Total number of cells counted}} \times 100$$

Statistical analysis

The data are presented as mean ± SD of the MN frequencies between control and different treatment groups and in between successive exposure periods. Data were analysed by one-way ANOVA followed with Bonferroni's Multiple Comparison post-hoc-test using SPSS (20.0) software. The accepted level of significance was $p<0.05$.

RESULTS AND DISCUSSION

Effect of anionic surfactant on induction of MN frequency

MN frequencies induced by anionic surfactant				
Groups	Exposure periods (h)			
	24	48	72	96
T1 (Cont)	0.15 ± 0.02^{a1}	0.17 ± 0.03^{a1}	0.11 ± 0.05^{a1}	0.09 ± 0.03^{a1}
T2 (LC_{50}/20)	0.2 ± 0.03^{a1}	0.27 ± 0.09^{b1}	0.33 ± 0.03^{b213}	0.39 ± 0.06^{b23}
T3(LC_{50}/10)	0.31 ± 0.08^{b1}	0.38 ± 0.05^{c1}	0.46 ± 0.04^{c213}	0.51 ± 0.05^{c23}

Values (means ± SD), with different alphabetical superscripts differs significantly ($p<0.05$) between different groups within exposure periods and numerical superscripts differs significantly ($p<0.05$) between exposure periods with the groups.

Among treated groups T2 and T3, group T3, is showing significant ($p<0.05$) changes in induction of MN frequency in comparison to control group T1 after 24h exposure period. However, both treated groups T2 and T3 were showing significant ($p<0.05$) changes in MN induction after 48h, 72h and 96h exposure periods in comparison to control group T1. Further, on comparing the treated group T3 with T2, the induction in frequency of MN was showing significant changes after 24h, 48h, 72h and 96h of exposure periods. Within the groups T2 and T3, the induction in frequency of MN was increased significantly ($p<0.05$) after 72h and 96h exposure periods on comparing with 24h exposure period and maximum MN frequency was recorded after 96h exposure period. The results obtained in this work are important in elucidating the genotoxicogenic properties of anionic detergent in fish. There are lot of literatures available for studies related to toxicity of detergents (Sobrino-figueroa, 2013). Detergents are mixture of different cationic and anionic surfactants, (Friedman and Wolf, 1996; Fatma *et al*., 2015) and additives such as: water softeners, preservatives, bleaching agents, pigments, enzymes, foam stabilizers, colorants and perfumes which may exert their additive and/or synergistic effects (Sorbino *et al*., 2013). *In vivo*, Surfactants affect cell membrane permeability and disturb the cellular metabolism (Argese *et al*., 1994). In addition, their metabolism generates ROS species leading to oxidative (Batool *et al*., 2010; Kannan and Jain, 2000; Shukla and Trivedi, 2018) and genotoxic stress (Javed *et al*., 2016; Tiwari *et al*., 2017). Furthermore, enzymes

present in detergent are extremely effective in aqueous solutions and long lasting (Jardak *et al*., 2016; Seddon *et al*., 2004), and excreting their toxic effects by instigating lysis of cell membranes (Ahyayauch *et al*., 2010).

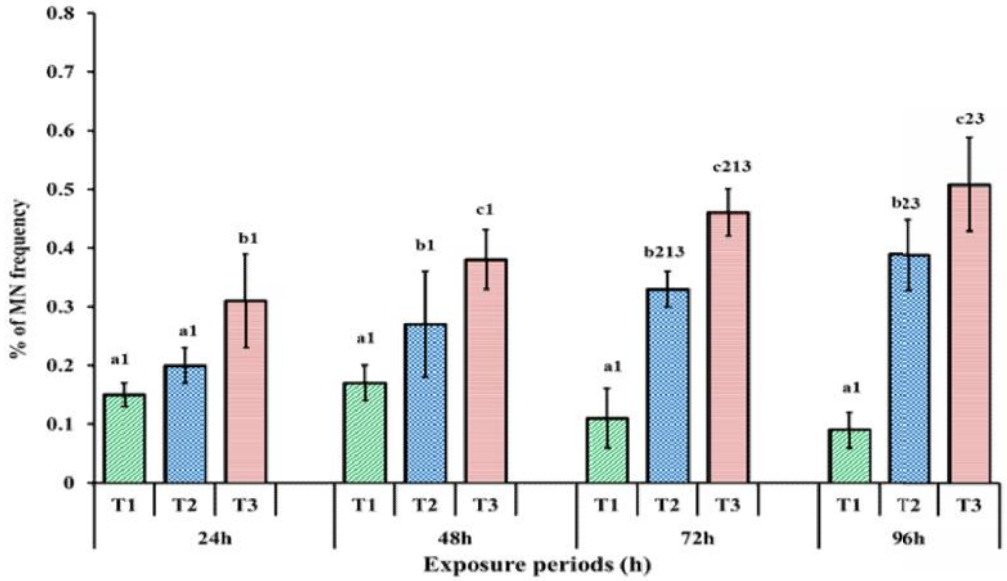

Fig. 1 *DNA damage in terms of MN frequency in control group (T1) and detergent treated groups (T2 and T3) in blood samples of fish C. punctatus after different 24h, 48h, 72h, and 96h exposure periods, The data represent mean ± SD of three independent experiments. (Values (means ± SD), with different alphabetical superscripts differs significantly (p<0.05) between different groups within exposure periods and numerical superscripts differs significantly (p<0.05) between exposure periods with the groups.).*

The MN induction is an eminent biomarker for assessing the genotoxicity in organisms exposed to toxic substances. The result obtained in the present study show a dose and exposure time dependent significant increase in the frequency of MN in fish erythrocytes after detergent treatment. The increase in the frequency of MN in fish erythrocytes recorded in this study is believed to be because of induction of ROS generated via oxidative stress and consequently, the disruption of the DNA repair process. Nieborowska-Skorska *et al*. (2009) have established the role of ROS in inducing micronuclei formation. Furthermore, it has been also established that detergent exposure causes oxidative stress by inducing ROS production (Nita and Grzybowski, 2016; Shukla and Trivedi, 2017) and consequently, causing genetic instability by inducing MNT formation (Abara *et al*., 2014).

Such findings verify the genotoxicity in fish *Channa punctatus* and prove that DNA damage along with nuclear abnormalities could be used as biomarkers in response to anionic detergent pollution load. It could also be used for early monitoring of freshwater bodies by using micronucleus assay to regulate population of this species in aquatic ecosystem.

Conflict of interest

Authors declare no conflict of interest**.**

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