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Induction of micronuclei in the erythrocytes of common carp, *Cyprinus carpio* (L) by caustic soda factory effluent

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Abstract

Studies concerning genotoxicity of industrial effluents are important in modern days. The present study was aimed to analyze the effect of mercury containing caustic soda effluent in fish which have -attracted many as laboratory animals for this type of study. Unfortunately, *Cyprinus carpio* are not suitable for chromosomal breakage studies because they have a high diploid number of chromosome ($2n=100$) which are small without marked variation in size. Recently, attempts have been made to examine the erythrocytes in fish for the occurrence of micronuclei (MN). In effluent exposed fish, a set time-response analysis demonstrated gradual decrease in the MN with an increase in exposure time. In the present study, concentration-response analysis showed the peak effect with 20% effluent at 24 hrs sampling periods, but with 21% at three sampling periods such as 48, 72 and 96 hrs respectively. Interestingly after 96 hrs exposure, no value was significantly higher than the control.

Keywords: Micronuclei, *Cyprinus carpio*, erythrocyte, Cuastic soda effluent and control.

1. Introduction

Industrial pollution has presently assumed global magnitude, due to indiscriminate discharge of effluents into the aquatic systems and the organisms inhabiting these water are always subjected to stress (Murugesan, 1988) [7]. Amongst pollutants, mercury and its compounds pollute aquatic habitats seriously and are extremely toxic to aquatic organisms Especially, to fish Jernelov, 1971 [6]; Sastry, 1982 [12] Murugesan *et al.*, 1997 [9]. In 1960s mercury poisoning was reported in Minimata Bay of Japan, where a number of persons died due to consumption of fishes contaminated with mercury (Fujuki, 1963) [4]. In aquatic ecosystem, fish species are the creatures that take higher place in food chain and are the significant food source for human beings. In addition to that in aquatic ecosystem, the fish could not escape from detrimental effects of various toxicants. This is the reason due to which fish are generally used as indicators of metallic ion pollution in aquatic habits (Agah *et al.*, 2009) [1]. As a indicator of genotoxicity, the formation of micronuclei in peripheral blood erythrocytes of fish was evaluated during the experiment. Moreover, selection of peripheral blood erythrocytes of fish as a target cell to investigate genotoxic change was based on the important role of blood in movement of toxic substances absorbed through skin, gills and digestive tract of fish species. Keeping in view the economic importance of Common carp *Cyprinus carpio* was selected to determine the genotoxic effects of caustic soda effluent on fish blood.

During the last few years, fish have attracted much attention as laboratory animals for micronuclei test study. Unfortunately, most of the fish are not suitable for chromosomes studies because they have high diploid number of chromosomes which are small and without marked variable in size (Ponnuraj *et al.*, 1998) [10]. Recently, attempts have been made to examine the peripheral erythrocytes in fish for the occurrence of micronuclei test (MNT) and using the information a monitoring system for potential genotoxicity of an agent proposed. The main advantage of this technique is that scoring of micronuclei is faster and easier than the conventional dicentric chromosome aberration technique (French *et al.*, 1988) [3]. Micronuclei are the product of broken or lagging chromosome which failed to become incorporated into either of the two daughter nuclei during cell division (Prosser *et al.*, 1989) [11].

In the present study, the incidence of micronuclei in the peripheral erythrocytes of *Cyprinus*

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carpio (L.) treated with caustic soda factory effluent was conducted; The aim of the research was to assess further suitability of micronuclei test (MNT) from the peripheral blood smear of fish for biological monitoring of environmental genotoxicants, and to detect a possible genotoxic effect of caustic soda factory effluent in a vertebrate model.

2. Materials and Methods

The fresh water fish *Cyprinus carpio* were procured from the government fish farm, Manimuthar, Tamil Nadu, India. Manimuthar river originates on the eastern slopes of Western Ghats in Tirunelveli district of the State Tamil Nadu in Southern India (8° 40 '57 N 77° 23' 15'' E). The fish were kept in tap water in the laboratory aquaria for one week for acclimation. The strong and active individuals with a body weight of 15-20 g were selected and allocated at random to various experimental groups (Murugesan and Haniffa, 1992)^[8]. The effluent of the Dharangadara Chemicals works Ltd, Arumuganeri, Tamil Nadu was collected from the discharge point, analysed for its constituents and acute toxicity studies were undertaken. A group of fish were released into different aquaria containing effluents of different concentrations and kept for varying periods of time (Table.1). Tap water was used

to dilute the effluent. The effluent was changed twice a day and aerated frequently. The animals kept in a tap water served as control. Four fishes were used for each sampling period and for each concentration level.

The fish in various groups were supplied with the same amount of pelleted feed. The control and effluent -treated fish were cut in the caudal region and smears of peripheral blood were made on grease-free clean slides. The smears were fixed in absolute methanol and air-dried, The next day, the slides were stained first in Giemsa and then in May-Grunwald and dried in air. The stained slides were observed under oil-immersion (Magnification 1000 X).

The frequency of cells with micronuclei in 2000 cells per individuals was determined and some cells were photographed. The non-refractile particles which resembled nuclei in all respects were considered to be micronuclei. Coded and randomised slides were scored by single observer (Schmid, 1976)^[12]. Frequency of micronuclei was calculated by using the following formula:

$$\text{MN frequency (\%)} = \frac{\text{Number of cells with Micronuclei}}{\text{Total number of cells counted}} \times 100$$

Table 1: Incidence of micronuclei in erythrocytes of common carp *Cyprinus carpio* exposed to caustic soda factory effluent

Concentration (% v/v)	Period of treatment			
	24 hrs MN frequency	48 hrs MN frequency	72 hrs MN frequency	96 hrs MN frequency
0	0	0	0	0
19.5	-	0.25 ± 0.10	0.19 ± 0.06	0.06 ± 0.06
20.0	-	0.69 ± 0.12	0.31 ± 0.16	0.25 ± 0.10
20.5	-	1.25 ± 0.40	0.44 ± 0.21	0.169 ± 0.12
21.0	-	0.50 ± 0.25	0.56 ± 0.06	0.44 ± 0.16
21.5	0.31 ± 0.24	0.31 ± 0.12	-	-

Values are mean ± S.E

3. Results

In 21% effluent the fish did not survive for more than 48 hours. Mortality in other concentrations of the effluent was nil. The erythrocytes of *Cyprinus carpio*, like those of other fish, are fairly large having centrally placed round nuclei and a sizeable cytoplasm. The size as well location of MN within the cytoplasm varied from cell to cell, the shape was round in almost all the cells. Each affected cell contained one micronucleus. In addition to the incidence of MN, a few erythrocytes exhibited some nuclear anomalies, e.g. some cells contained two normal nuclei (i.e., they were binucleate); in some cells the micronucleus was connected to the main nucleus by chromatin bridge; in some cells there was bilobed nucleus or normal nucleus showing an eroded appearance

(Fig.1).

The frequencies of MN in the effluent treated individuals showed distinct concentrations dependent as well as time-dependent. Table.1 summarises the incidence of induction in the peripheral MN erythrocytes of fish exposed to different concentrations of the effluent and tap water (as control). In effluent-exposed fish, a set time-response analysis demonstrated gradual decrease in the MN with an increase in the exposure period. Concentration-response analysis showed the peak effect with 20% effluent at a 24 hours sampling period, but with 21% at 48 hours, 72 hours and 96 hours sampling periods respectively. Interestingly, after 96 hours exposure, no value was significantly higher than the control

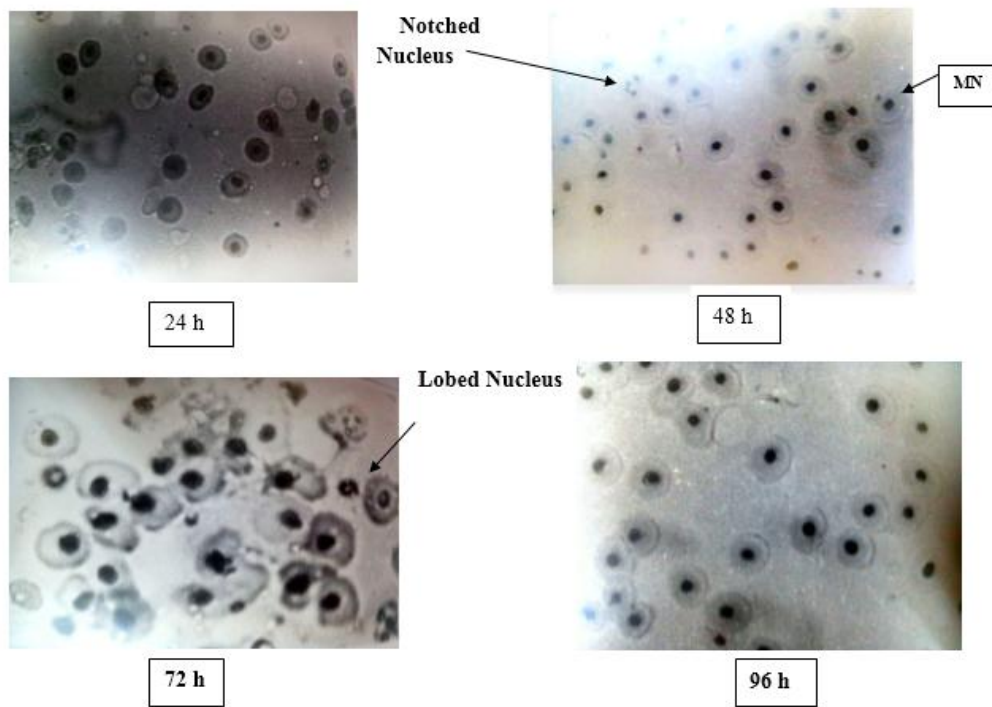


Fig 1: Erythrocytes of common carp *Cyprinus carpio* (L.) showing micronuclei induced by caustic soda factory effluent at different exposure such as 24 h, 48 h, 72 h and 96 h respectively

4. Discussions

The induction of MN is caused by both clastogens and spindle poisons (Schmid, 1976) [12]. The occurrence of lower frequency of micronucleated erythrocytes than that of chromosome aberration of the treated specimens referred above could be explained in the light that all forms of aberrations could not contribute to the formation of MN. So, in the present study the MN might be produced by the combined clastogenic and spindle poisoning effects of the effluent. The gradual decrease in the frequency of MN with an increase in exposure periods as well as at a higher concentration can be explained by the inhibitory effect on the cell division and subsequent hindrance to the passage of the affected cells into the peripheral circulation. Formation of these anomalies in fish blood clearly indicate that caustic soda effluent has high genotoxic potency and insight of socio-economic purpose; it is very harmful for aquatic ecosystem. However, the well-documented and reasonable significant associations with an increase the occurrence of MN and exposure periods are still unknown (Bolognesi *et al.* 2006) [2]. Although the significant increases in the erythrocyte MN in fish after 48 hours recorded whereas some workers have demonstrated the increased frequencies of MN at 72 hours was also reported by Zhu *et al.*, 2009 [15] in *Cyprinus carpio* due to exposure of copper. These variations may also be a direct consequence of the sensitivity behaviour and the niche of the species, as the number of MN in the cells of fish may be variable (Gvaziela *et al.* 2010) [4]. Micronucleus test using peripheral blood smear of fishes could serve as a very useful quick mutagenicity testing protocol throughout the year in detecting the clastogenic agents in aquatic environments (Sobti and Kaushal, 1997) [14]. It would be advantage to examine a number of cells even in species which are unsuitable for chromosome aberration study for adverse karyotype and chromosome size, structure and number. The number of dividing cells for chromosome study might not be adequate throughout the seasons.

5. Conclusions

It is evident that multiple mechanisms can lead to the formation of MN. MN frequencies provides a useful idea of accumulated genetic damage during the lifespan of cells. The MN frequencies in blood evaluate the kinetic of cytogenetic alterations under influence of caustic soda effluent. Therefore, the extensive release of these effluents in the water bodies and its surface area should be avoided.

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