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# CYTOGENETIC EFFECT OF CHROMIUM TRIOXIDE IN AN AIR BREATHING TELEOST CHANNA PUNCTATUS (BLOCH)

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**ABSTRACT**

*Chromium can damage DNA in several ways, including DNA double strand breaks (DSBs) which generate chromosomal aberrations, micronucleus formation, sister chromatid exchange, formation of DNA adducts and alterations in DNA replication and transcription. In our study, fishes (Channa punctatus) were exposed to five sub-lethal concentrations of chromium trioxide viz. 1.28, 1.60, 2.14, 3.21, 6.42 mg/l for 24, 48, 72 and 96 h. Statistically significant increase in the frequency of micronucleated erythrocytes, as compared to negative control, in the peripheral blood of chromium treated fish suggest its genotoxic effect.*

**KEYWORDS:** *Genotoxicity, Chromium, Fish, Micronucleus.*

## INTRODUCTION

Among all types of aquatic pollutants, heavy metals are of greatest concern because after reaching in the aquatic bodies they deteriorate the life sustaining quality of water. Among the heavy metals, chromium is one of the major aquatic pollutants in many parts of the world. Chromium has been listed as one of the 129 priority pollutants and considered one of the 14 most noxious heavy metals. Chromium compounds are known to have genotoxic, mutagenic and carcinogenic effects on man and animals [1, 2]. Chromium enters the environment through natural and as well as various anthropogenic sources. This metal occurs naturally in rocks, soil and water. Chromium is widely used in industries for production of chromates, refractory materials, chromium steels, ferrochromium, cement, fungicides, pigments, oxidants, catalysts, fertilizers etc. and released into aquatic environments mainly by electroplating, tannery and textile industries [3]. Heavy metals produce deleterious effects on aquatic flora and fauna by affecting various physiological, biochemical and cellular processes [4-5]. Due to its redox potential, hexavalent chromium (Cr (VI)), can induce oxidative stress in fish. Reactive oxygen species (ROS) such as $O^{\cdot}_2$, $H_2O_2$, $OH^{\cdot}$ plays a key role in chromium induced toxicity leading to the production of and subsequent modulation of the activities of antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), metallothioneins (MT), glutathione proxidase (GPx), genotoxicity and histopathology [6]. Various fishes have been investigated in relation to heavy metal toxicity [7-10]. The main aim of the current study was to assess the genotoxic effect in the Channa punctatus

exposed to chromium trioxide The evaluation was made through analysis of the MN frequencies in peripheral erythrocytes.

## MATERIALS AND METHODS

*Channa punctatus* was selected as the test species for present investigations because of its abundance and ready availability throughout the year. Healthy specimens (12-16 cm length and 50-55g weight) were collected from a single population from local fish market and were treated with 0.05% $KMnO_4$ solution for 2 minutes to avoid any dermal infection. Fish were acclimatized for 15 days in the laboratory in glass aquaria containing 30 l non-chlorinated tap water. For genotoxicity assay five concentrations of chromium trioxide ($CrO_3$, CAS No. 1333-82-0, M.W 99.99. Loba Chemie, Mumbai) namely 1.28 (1/50 of LC50), 1.60 (1/40 of LC50), 2.14 (1/30 of LC50), 3.21 (1/20 of LC50) and 6.42 (1/10 of LC50) mg/l were selected on the basis of 96 h LC50 concentration. Acclimated fishes were treated for 24, 48, 72 and 96 h. Positive control fishes were treated with Cyclophosphamide @ 5mg/l for the same period.

For micronucleus assay blood was collected from caudal vein using cold hypodermic syringes pre-rinsed with heparin (anticoagulant). The collected blood from the control and experimental groups was expelled on clean glass slides and thin smears were prepared. The slides were air-dried for 24 h, fixed in methanol for 10 minutes, and stained in 10% Giemsa (v/v). The erythrocytes were examined for the presence of micronuclei in their cytoplasm. Micronuclei were identified as small (diameter less than one-third of the main nucleus) non-refractive, circular or ovoid chromatin bodies separated from the main nucleus and have similar staining as the main nucleus

Data are expressed as mean ± SD and analyzed by Dunnet multiple comparisions test to test the significance of difference between control and treated animals. Analysis of variance (ANOVA) test was conducted to determine the significance of the effect of treatment as well as periods of treatment on frequency of micronucleated erythrocytes by Graph Pad Prisma V.3 computer programme.

## RESULTS

Frequencies of micronucleated erythrocytes recorded in fish treated with chromium trioxide as well as control and positive control fishes. It is evident from the **table-1** that treatment of fish with chromium chloride resulted in concentration and period of treatment related increase in the frequency of micronucleated erythrocytes in peripheral blood when compared to untreated control fish. The differences between mean number of micronucleated erythrocytes of chromium trioxide treated fish and control fish were statistically significant at 1.28 mg/l (after 72

and 96 h 0f treatment), 1.60 mg/l (after 48, 72 and 96 h of treatment) and at 2.14, 3.21 and 6.42 mg/l (after 24, 48, 72 and 96 h of treatment). **Figure-1** illustrates the trend of increase in the frequency of micronucleated erythrocytes. It is clear that the frequency increased almost linearly with increase in concentration of chromium trioxide. Two-way analysis of variance test (ANOVA test) revealed highly significant ($p<0.001$) difference between the concentrations of chromium trioxide as well as periods of treatment (**Table 2**).

**Table 1 Frequency of micronucleated erythrocytes in blood of freshwater fish *Channa punctatus* treated with different concentration of chromium (VI)**

| Chemical / Concent | Frequency (%) of micronucleated erythrocytes[a] | | | |
|---|---|---|---|---|
| ration | 24 h | 48 h | 72 h | 96 h |
| Control | 0.024 ± 0.016 | 0.022 ± 0.030 | 0.030 ± 0.020 | 0.019 ± 0.011 |
| CP | 0.36 ± 0.092** | 0.048 ± 0.135** | 0.59 ± 0.135** | 0.68 ± 0.082** |
| 1.28 mg/l | 0.062 ± 0.016 | 0.108 ± 0.024 | 0.126 ± 0.036* | 0.162 ± 0.044** |
| 1.60 mg/l | 0.082 ± 0.028 | 0.110 ± 0.018* | 0.150 ± 0.020* | 0.210 ± 0.030** |
| 2.14 mg/l | 0.110 ± 0.030** | 0.164 ± 0.028** | 0.185 ± 0.028* | 0.25 ± 0.026** |
| 3.21 mg/l | 0.165 ± 0.025** | 0.190 ± 0.0.32** | 0.222 ± 0.034** | 0.31 ± 0.042** |
| 6.42 mg/l | 0.194 ± 0.028** | 0.220 ± 0.042** | 0.250 ± 0.048** | 0.38 ± 0.038** |

[a]**1000 cells per fish and total 5000 cells have been scored in each case**

*** ($p<0.05$) and ** ($p<0.01$) differ significantly from the control in Dunnet multiple comparision test.**

**Table 2 Two-way analysis of variance (ANOVA) of mitotic index showing significant variation between treatments as well as periods of treatment**

| Sources of Variation | df | Mean Square | F-value |
|---|---|---|---|
| Between periods | 3 | 0.0376 | 13.42*** |
| Between treatment | 5 | 0.0564 | 20.14*** |
| Residual | 15 | 0.0028 | |

***** Significant at $p<0.01$ and $<0.001$ respectively**

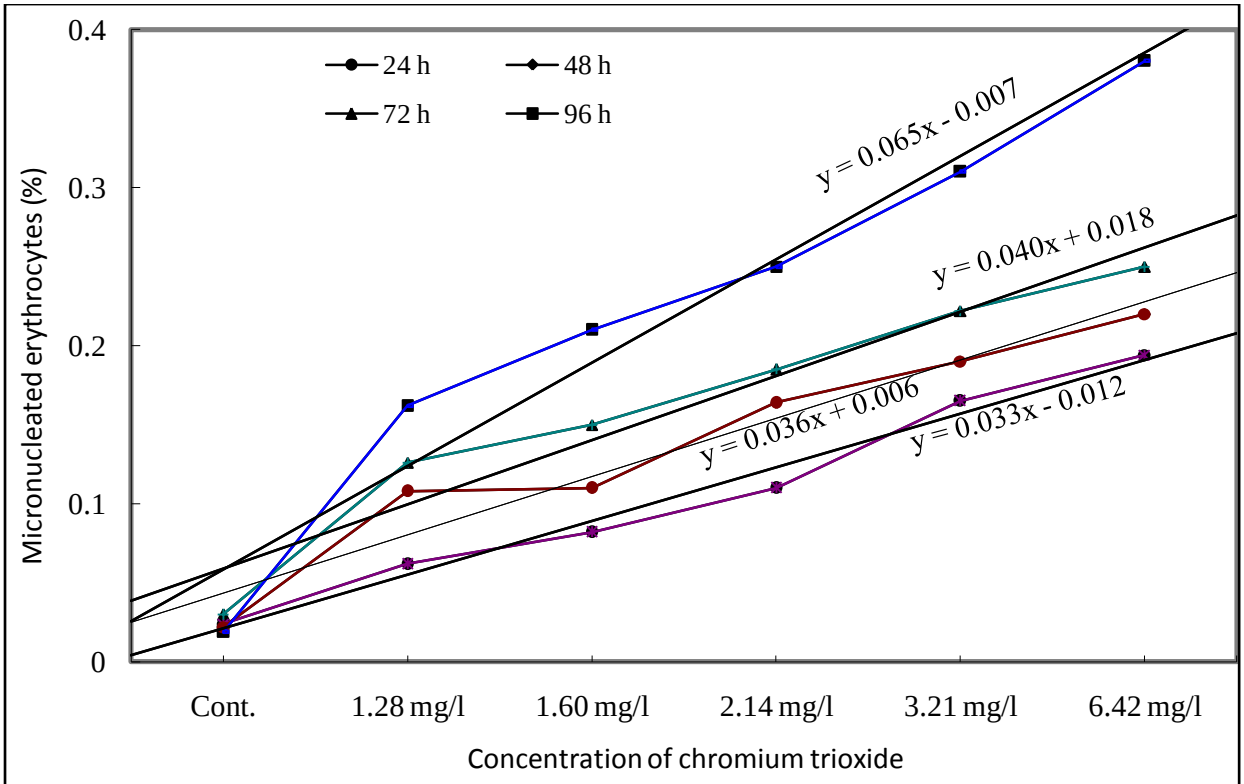


**Fig. 1: Trends of increase in the frequency of micronucleate erythocites in the peripheral blood of fish after chromium trioxide treatment**

## DISCUSSION

In recent years, cytogenetic investigations for genotoxicity monitoring of water contaminants by employing fish has gained enhanced interest [11-18]. These studies have clearly demonstrated the utility of fishes as indicators for the monitoring of environmental carcinogens, teratogens and mutagens. Various studies reported cytotoxic, immunological, hematological, and histological and genotoxic effects of chromium in fish. Both trivalent chromium III (Cr III) and hexavalent chromium VI (Cr VI) are biologically active Chromium (VI) is a strong oxidizing agent, diffuses readily in the tissues and can easily penetrate cell membranes [19]. The toxic action results from its strong oxidative effect on membrane phospholipid proteins and nucleic acids [20]. Al-Sabti [21] reported significant increase in frequency of micronuclei in erythrocytes of *Carassus auratus gibelio* fish exposed to sub-lethal chromium concentrations in the laboratory and fish collected from chromium-contaminated rivers. De Lemos et al [11] showed significant induction of micronucleated erythrocytes in fishes exposed to chromium (VI) for 7, 14 and 21 days and the induction was found to be decreased after 21 days of exposure. A significant increase in chromosomal

aberrations was observed after 72 hrs of [Cr(VI)] exposure of *C. punctata* [22]. Sodium chromate induced DNA double-strand breaks in medaka cells and also chromosomal aberrations causing chromatid lesions and exchanges [23]. Several in vivo and in vitro studies have shown that chromium compounds damage DNA in a variety of ways, including DNA single and double-strand breaks generating chromosomal aberrations, micronucleus formation, sister chromatid exchanges, formation of DNA adducts, and alteration in DNA replication and transcription [24-26]. It has been suggested that genotoxic activity of chromium occurs through its intracellular reduction from chromium (VI) to chromium (III), the most stable form of chromium ion and reactive intermediates such as chromium (IV) and chromium (V). During reduction process, oxygen generated as highly reactive free radical species that can react with DNA [27-28]. The micronucleus assay in the hematopoietic cells is one of the most sensitive tools to evaluate the genotoxic property of water contaminants. The test is being applied for both *ex situ* and *in situ* monitoring of genotoxic effects due to exposure to environmental pollution. The results of the present study indicate that exposure of fishes to chromium trioxide resulted in concentration and period of treatment related increase in the frequency of micronucleated erythrocytes. Micronuclei are formed due to condensation of acentric chromosome fragments or lagging chromosomes that fail to incorporate into daughter cell nuclei during cell division. Therefore, cytogenetic damage that results in chromosome breaks or spindle abnormalities leads to micronucleus formation and thus the incidence of micronuclei serves as an index of genotoxic effect. The obtained results on micronucleated erythrocytes in the peripheral blood of fish *(C. punctatus)* revealed that chromium trioxide induced clastogenic and aneugenic effects and primary DNA damage in blood cells of exposed fishes.

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