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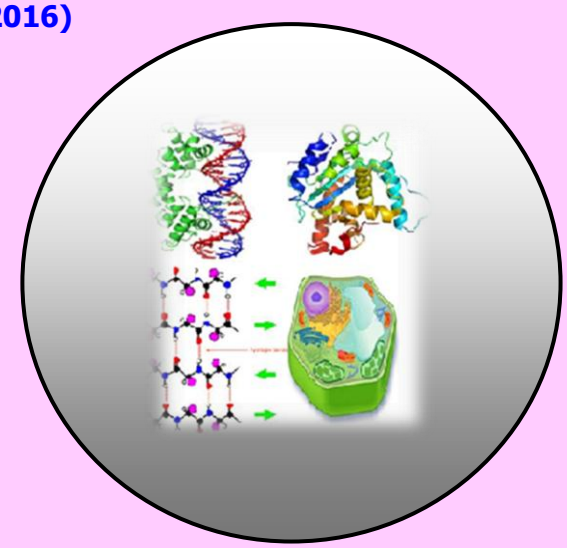

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RESEARCH PAPER

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In vitro Study of Genotoxicity of 2,4-D in Fresh Water Fish *Channa punctatus*

***Abdul Bari Khan, Jayashree Singh, **Cherish Sheba Nura, Anurag Kumar, Kumari Sukshma and Dhyanendra Kumar**

***Dr. Z H.I.I. College Nidhauli Kalan Etah, U.P., 207122, India**
****Department of Chemistry Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad, U.P., India**
Department of Zoology Veer Kunwar Singh University, Ara, Bihar, 802301 India

ABSTRACT

Herbicide usage in agriculture field to control weeds is toxic to non-target organisms like fish and affects fish health, impairment of metabolism, sometimes leading to mortality. In the present study the changes in genetic material by 2,4-Dichlorophenoxy acetic acid(2,4-D) using micronuclei test in fish Channa punctatuswas measured. Changes in the genetic material due to the effect of genotoxic chemical generally represent the first step of the process of chemical mutagenesis. The differential sensitivity of species to herbicide was evaluated by exposing individuals to different doses of 2,4-D in different time period i.e. 24 hr, 48hr, 72hr or 96hr. we organism was sensitive to the herbicide and fraction of micronuclei noticed gradually.

Keywords: Genotoxicity, Herbicide 2,4-D, Micronucleus, Channa punctatus.

INTRODUCTION

Currently a range of biomarkers and bioassays are in practice in the laboratory and field studies to determine the effects of genotoxic pollution. The presence of DNA adducts, chromosomal aberrations, DNA strand breaks and measurement of micronuclei frequencies are commonly considered criterion. Among the currently available test systems micronucleus test is widely used as a cytogenetic method to assess chromosomal damage induced by various genotoxicantsdue to its simplicity, reliability, sensitivity and proven suitability for fish species.

Evaluation of micronucleus frequency studied by De Lemous*et al.* (2001). The formation of micronuclei development on proliferation of cell depends on fish species, target tissues and kind of pollutions involved. The micronuclei cells formed spontaneously in fish cells worked

by William and Metcalfe (1992). However, the clastogenic effects of pollutants can be measured in different target tissue such as erythrocytes, gill, kidney, liver etc (Arslan and Parlak; 2017 and Woodward and Directorate, 2017). Hayashi *et* al. (1998) Defloraet *al.* (1993) Das and Nanda (1986) noted an increase in micronuclei frequency in the erythrocytes of fossils exposed to mitomycin and paper mill effluent. Mehdi et *al.,* (2011) studied the toxicity in agricultural soil. Abul Farah *et al.* (2002) evaluated the genotoxic effect of pentachlorophenol and 2,4-D in fish *Channa punctatus*. Shao-nan *et al*. (2007) observed exposure of sub-lethal doses of 2,4-D on different time durations. Murthy *et al.,* (2013) shows disruption of internal organ. Devi & Mishra (2013) detect the biotranformation, genotoxic and histopathological effect of environmental contaminants in fishes (Gibbons *et al.,* 2015). The Increasing human population and industrial development has worsend problem of disposal of anthropogenic chemicals and waste in aquatic environment. Large portions of these contaminants are potentially genotoxic and carcinogenic substances. Agents that produce alternation in the nucleic acids and associated components of sub-toxic exposure levels, resulting in modified hereditary characteristic or DNA inactivation, are classified as genotoxins. These chemicals are responsible for DNA damage in variety of aquatic organisms and fishes in particular, causing malignancies, reduced growth, abnormal development, reduced survival of embryo ultimately affective the economy of fish production significantly. Genetoxicity, not only reduces the fitness in wild fish population, but also pose risk to human health via food chain. 2,4-Dichlorophenoxy acetic acid, commonly called 2, 4-D is common systemic pesticide/herbicide used to control broad leaf weeds. It is one of the most widely used herbicide in the world. It is synthetic auxin, and as such it is often used in laboratories for plant research. Genetic toxicology investigates the interaction between chemical and physical agents with genetic materials which shows subsequent adverse effect, such as cancer or genetic disease in further generation. Micronucleus test is *invitro* biomarker assays which have been used for assessment of genotoxic and mutagenic effect of environmental pollutants. Micronucleus test is also a simple and sensitive assay, as defined as cytoplasmic chromatic masses that look like small nuclei or they arise from lagging at anaphase or from acentric chromosomal fragment or entire chromosome. In present study, attempts were made to investigate the genotoxic effect of 2,4 –D using micronucleus assay in erythrocyte.

MATERIALS AND METHODS

Test Organism

The fish that has been selected for the present study is fresh water teleost fish *Channa punctatus.* The used fish was procured locally from fish market. 11-13 cm long and weighing 30-35gm. In the laboratory the diseased fish were discarded and healthy fishes were selected for experiment. The selected fishes were treated with 0.5% $KMno_4$solution for 2 minutes to avoid any dermal infection. Feeding of the fish was stopped 24 hrs before the experiment. Every effort as suggested by Bennett and Booley (1982) was made to9 maintain optimal conditions during acclimatization. 2,4-D used as test chemical and experiment was done on three different concentration i.e. 195 ppm, .381 ppmand .58 ppm

Micronucleus (MN) assay - Micronucleus test in erythrocytes of *Channa punctatus* was done according to (Kushwaha *et al.* 2000 and Ali *et al.* 2008). The micronucleus test is a simple and sensitive assay for in vitro evaluation of genotoxic properties of various agents.

At first blood is collected and immediately after blood collection the slides were prepared by smearing one drop of blood on a clean microscopic slide. The smears were fixed in methanol for 10 min and left with 6% Geimsa for 20 min. A total of 2000 erythrocytes were examined for each sample under the light microscope. The criteria for the identification of micronuclei used were no connection with the main nucleus, and area smaller than one third of main nucleus.

Scoring of Micronucleus (MN) slides- Uniform criteria for scoring micronucleus should be followed. Only micronucleus not exceeding 1/3 of the main nucleus diameter , and a total of 1000 erythrocytes cells were scored from each slide for presence of micronuclei, criteria for scoring micronuclei were adapted from Fenech*et al.* (2003).

The micronucleus frequency was calculated as under

$$MN\% = \frac{\text{No of cells containing micronucleus}}{\text{Total no of cells counted}} \text{ X } 100$$

Statistical analysis- The percentage of micronucleus frequency in different exposure time and concentrations were compared using the Mann-Whitney test using SPSS software (Standard Version 11.5 SPSS Inc.). Pvalue less than 0.05 was considered statically significant.

RESULTS

The result of Micronucleus analysis in erythrocytes of *Channa punctatus* at different concentrations and durations indicated significant induction ($P<0.05$) of micronuclei, the fish specimens due to 2,4-D exposure than compared to the control group (Table-1.) Micronuclei induction was significantly higher in the positive than the negative control from 24 hr.to 96 hr. Increase in the concentration of the herbicide resulted in higher induction of micronuclei with the highest frequency recorded at SL-III (.58 ppm) on 96 hr (0.268) followed by SL-II (0.35 ppm) and SL-I,(0.19 ppm) the micronuclei formation was highest (0.183) and (0.157), respectively .

DISCUSSION

There was a gradual nonlinear increase in micronuclei frequency for all the concentrations. Criteria for identifying a positive response (i.e. increased frequency of micronucleated cells in an exposed group compared to the different population, a dose response relationship if variable exposure levels have been established) must be identified prior to implementing the study. A positive result is supported by a dose response relationship when individuals with different exposure levels are considered. A dose response relationship cannot however be a pre-requisite; an increased micronuclei frequency may reflect an effect of a long term exposure for which accurate dose estimation is difficult. Shah *et al.* (2005) explain the maximum confidence that the exposure results in an increased frequency of micronuclei requires reproducible results in independence studies. The lack of a statistically significant increase in micronucleated cells indicates that under the exposure conditions evaluated and for the calculated power of the study, the exposure did not result in a significant increase in chromosomal damage in a cell population evaluated.

In the present study all concentrations of 2,4-D induced significantly (P<0.005) higher number of micronuclei than the control and its frequency increased with concentrations and durations.

Through the result, the micronucleus assay showed that concentrations and durations both are effective in induction of micronuclei and DNA damage due to 2,4-D.

CONCLUSION

In the present study significant differences were observed *in vitro* with regard to percentage of Per cent MNi frequency following exposure to 2,4-D over a range of concentrations and durations. Thus the micronucleus assay can be used in combination for screening genotoxic effect of chemicals and for examining the implications of DNA damage and its recovery in the sentinel fish species. These biomarkers have also opened a broad perspective in aquatic toxicology as fish erythrocytes is constantly being exposed to environmental pollutions.

Table 1. Introduction of micronuclei (MN) in peripheral erythrocytes of *Channa punctatas* exposed to different concentrations of 2,4-D.

Exposure Time	Concentration	No. of Fishes	No. of Observed	No. of Cells observed	MN % Frequenc-es ± SE
24 hrs	Control	4	8227	296	0.036 ± 0.023
	Positive Control	4	8318	400	0.048 ± 0.018
	SL-I	4	8121	496	0.061 ± 0.023
	SL-II	4	8209	696	0.085 ± 0.012
	SL-III	4	8265	996	0.121 ± 0.032
48hrs	Control	4	8265	396	0.048 ± 0.015
	Positive Control	4	8437	412	0.049 ± 0.017
	SL-I	4	8340	700	0.084 ± 0.023
	SL-II	4	8312	996	0.012 ± 0.023
	SL-III	4	8296	1200	0.0145 ± 0.053
72 hrs	Control	4	8362	260	0.036 ± 0.012
	Positive Control	4	8241	796	0.097 ± 0.020
	SL-I	4	8425	892	0.106 ± 0.040
	SL-II	4	8523	1048	0.152 ± 0.061
	SL-III	4	8376	1500	0.179 ± 0.023
96 hrs	Control	4	8367	300	0.036 ± 0.223
	Positive Control	4	8294	1200	0.145 ± 0.034
	SL-I	4	8279	1300	0.157 ± 0.064
	SL-II	4	8187	1496	0.183 ± 0.030
	SL-III	4	8180	2192	0.268 ± 0.075

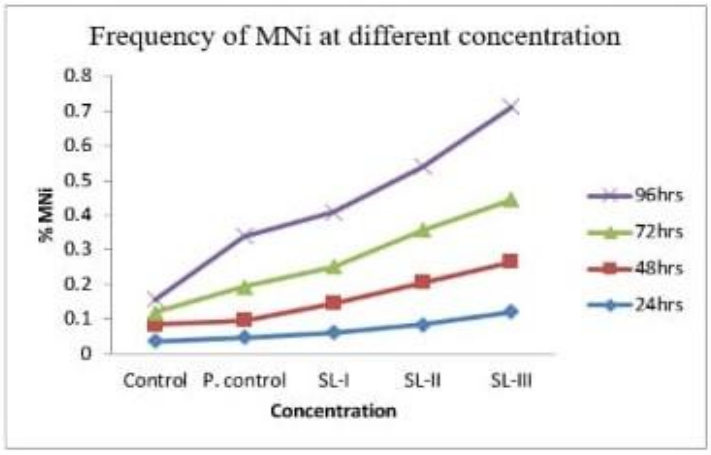

Figure 1. Variation of Micronuclei Frequency (a). SL-I (b). SL-II and (c). SL-III of concentration of 2,4-D in relation to different exposure time.

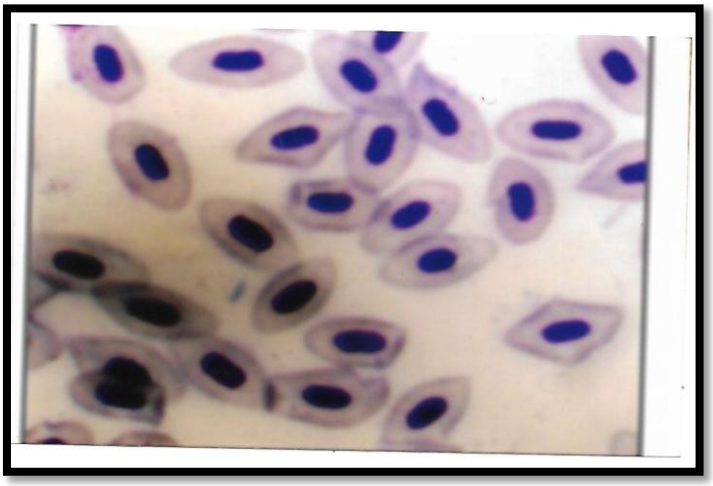

Figure 2. Micronuclei induction – control cell showing no micronuclei.

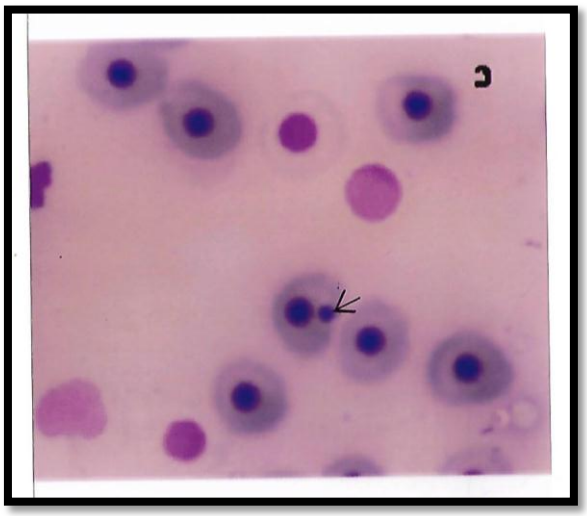

Figure 3. 2,4-D exposed blood showing micronuclei on 24 hrs at 0.19 ppm.

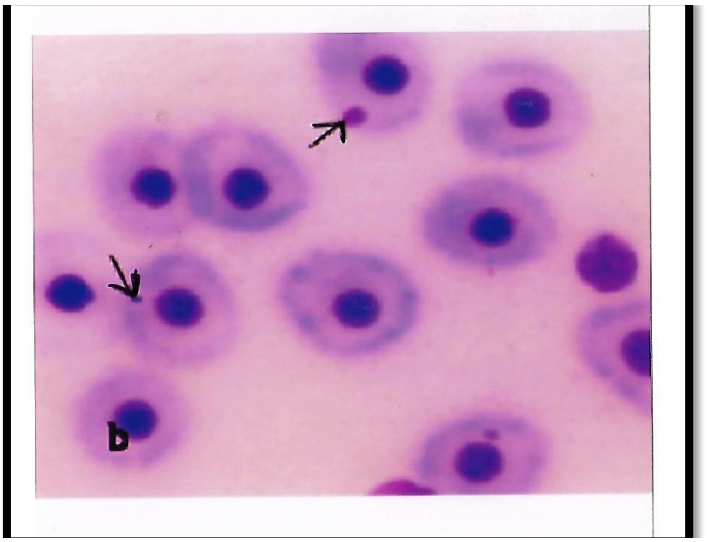

Figure 4. 2,4-D exposed blood showing micronuclei on 48hrs at 0.35 ppm.

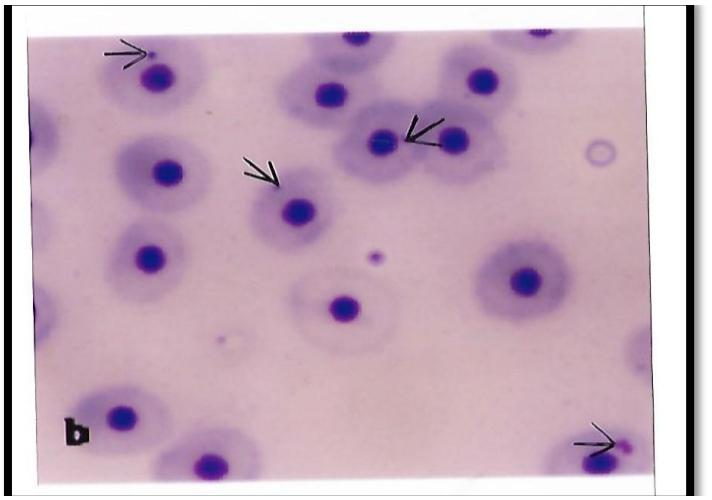

Figure 5. 2,4-D exposed blood showing micronuclei on 72 hrs at 0.58 ppm.

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Corresponding author: Jayashree Singh, Department of Zoology Veer Kunwar Singh University, ARA, BIHAR, 802301 INDIA.
Email: jsrisingh6@gmail.com, profdkumar@gmail.com