WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 7, 902-908. Research Article ISSN 2277– 7105

EVALUATION OF MICRONUCLEI AS GENOTOXIC ASSAYS INDUCED BY DRUG DICLOFENAC IN THE FRESH WATER FISH, *CIRRHINUS MRIGALA*

S. Binukumari[1], V. Priyadarsini[2] and J. Vasanthi*[3]

[1, 2]P. G and Research Department of Zoology, Kongunadu Arts and Science College, Coimbatore.

[3]PG and Research Department of Zoology, Nirmala College for Women, Coimbatore.

Article Received on 04 May 2016,
Revised on 25 May 2016,
Accepted on 16 June 2016
DOI: 10.20959/wjpr20167-6485

***Corresponding Author**
J. Vasanthi
PG and Research
Department of Zoology,
Nirmala college for women,
Coimbatore.

ABSTRACT

Fishes belonging to the species *Cirrhinus mrigala* were exposed to 2.8 per cent concentration of Diclofenac drug for 24, 48, 72, 96 hours and 10 days, 20 days and 30 days respectively. Bioassay studies were conducted to calculate 50 per cent mortality of *Cirrhinus mrigala* in 96 hour by exposing to different concentrations of Diclofenac drug. Blood was punctured from the gill and analysed for genotoxicological test. Among the different exposure periods, the frequency of micronuclei higher in 30 days exposure period compared to the control.

KEY WORDS: *Cirrhinus mrigala,* genotoxicological.

INTRODUCTION

Pharmaceuticals and PCPs eventually get washed from the body and enter water systems, ultimately winding up in the effluent of wastewater treatment plants and aquatic environments. Since medical substances are developed with the intention of performing some sort of biological function, they have a tendency to bioaccumulate and induce effects in aquatic and terrestrial ecosystems (Halling-Sorensen *et al*., 1998). Fishes are appropriate as environmental toxicity bioindicator organisms, both due to their role in the aquatic tropic chain and because of their sensitivity to low concentrations of toxic substances.

Several studies have looked at the toxic and genotoxic action of aquatic environments at industrial, urban and rural activities using a variety of analytical methods, bioassays and biomarkers both in vitro and in vivo techniques (Lemos and Erdtmann, 2000; Pacheco and

Santos, 2002). Fishes are appropriate as environmental genotoxicity bioindicator organisms, both due to their role in the aquatic trophic chain and because of their sensitivity to low concentrations of genotoxic substances, characteristic of polluted aquatic environments (Lemos *et al*., 2007).

Genotoxic effects on fish can be evaluated by a number of techniques, especially micronuclei analysis in peripheral erythrocytes (MNE) and cytogenetic analysis. These provide a quick, sensitive and reliable analysis to determine the damage. Yadhav and Trivedi, (2006) evaluated the genotoxic potential of Chromium (VI) in *Channa punctata* fish in terms of chromosomal aberrations. The remarkable chromosomal aberrations record chromatid breaks, chromosome breaks, chromatid deletions, fragments, acentric fragments and dicentric chromosomes along with chromatid and chromosome gaps.

MATERIALS AND METHOD

Cirrhinus mrigala is the freshwater carp found in Northern India, Punjab, West Bengal and Orissa. It has a wider mouth and thinner lips. Body silvery, dark grey alomg with the back, sometimes with coppery tinge. Adult attains a maximum length of 90 – 100 cm and a weight of 1.4 to 2.8 kg. The growth of about 20 cm is recorded in 8 months. This growth data is based on an experiment conducted on 6000 fingerlings.

ANALYTICAL TEST FOR WATER CHEMISTRY

The tap water free from contaminants was used as dilution water for the present study. The physico – chemical analysis of water used in the experiments was carried out using the methods of APHA, (2005).

Physico – chemical parameters of the tap water used for the present study are as follows: Temperature 27.2 ± 0.9 (0C), pH 7.1 ± 0.1, Dissolved oxygen 5.4 ± 0.4 (mg/l), Calcium 120 ± 1.1 (mg/l), Magnesium 50 ± 0.2 (mg/l).

COLLECTION AND MAINTENANCE OF FISH

The fingerlings of the freshwater fish, *Cirrhinus mrigala* ranging in weight from 4 kg to 8 kg and measuring (4 cm to 6 cm in length) were procured from Aliyar. The procured bulk samples of *Cirrhinus mrigala* were transported to the laboratory in well aerated polythene bag and acclimatized to the laboratory conditions under natural photo period for one week in large plastic containers at (26 ± 5 ^{0}C). The tank was previously washed with potassium

permanganate to prevent any fungal infection. The fishes were maintained in dechlorinated tap water of the quality used in the test and water was renewed every day to provide freshwater rich in oxygen.

Continuous artificial aeration was maintained throughout the acclimation and exposure periods. During the periods of acclimation they were fed everyday with oil cake mixed with rice flour. Unhealthy fish and those with infections were removed. Feeding was stopped two days prior to the experiment to maintain same state of metabolic requirements. Fish belonging to both sexes were selected for the present investigation. All the precautions laid down on recommendations of the toxicity tests to aquatic organisms are followed Anon, (1975).

TOXICANT

Analytical grade Diclofenac 2 [(2,6 – Dichlorophenyl) amino] benzene acetic acid sodium salt [DCF, 99.9% pure]; CAS No.15307-79-6 were purchased from Sigma-Aldrich chemic GmbH, Germany, Dimethyl sulphoxide (DMSO) (CAS No 67-68-5) was purchased from Fischer Scientific India Pvt. Ltd, India and 0.2 ml/l used to prepare the stock solution at different concentrations (1, 10 and 100 µg/L due to their low water solubility. IUPAC Name is 2 [(2, 6 – Dichlorophenyl) amino] benzene acetic acid sodium salt. Molecular formula is $C_{14}H_{10}C_{12}NNaO_2$ and molecular weight is 381.13.

EVALUATION OF MEDIAN LETHAL CONCENTRATION

The concentrations of the pollutant at which 50 percent of the test animals die during a specific test period of time is referred to as median lethal concentration (LC_{50}) or median tolerant limit. In aquatic toxicology the traditional LC_{50} test is often used to measure the potential risk of a chemicals (Jack de Bruijin *et al*., 1991).

Batches of 10 healthy fishes were exposed to different concentrations of drug, Diclofenac to calculate the LC_{50} value. One more set of fishes are maintained as control in tap water. To find the wide range of concentration 100-600 ml were chosen and the number of dead or affected fishes was counted at regular intervals upto 48 hrs. The level of the dissolved oxygen, pH, alkalinity and hardness were monitored and maintained constant.

Appropriate narrow range of concentration was used to find the median lethal concentration, using a minimum of 6 fishes for each concentration and the mortality was recorded for every

24 hrs upto 96 hrs. It was found as for 96 hrs, using probit analysis method (Finney, 1971). From the stock solution various sublethal concentrations were prepared for bioassay studies. Three groups of fishes were exposed to 1/10 of the drug 'Diclofenac' for 24, 48, 72, 96 hrs, 10 days, 20 days and 30 days respectively. Another group was maintained as control. All the groups received the same type of food and other conditions were maintained similarly. At the end of each exposure period, fishes were sacrificed and tissues such as gill, liver and kidney were dissected and removed. The tissues (10 mg) were homogenized in 80% methanol, centrifuged at 3500 rpm for 15 minutes and the clear supernatant was used for the analysis of different parameters.

GENOTOXICOLOGY

MICRONUCLEUS ASSAY

Micronucleus test was performed as per method of Schmid (1975). Blood from fishes of each group was collected from heart puncture in a heparinised syringe to make a thin smear on precleaned slide. Slides were fixed by dipping it in absolute methanol for 5-10 min, air dried for atleast one hour and stained with Giemsa for 10 mins. Slides were washed with distilled water air dried overnight, mounted with DPX and observed under Nikon microscope using 40/100 x objective lenses and were scored for micronucleated cells.

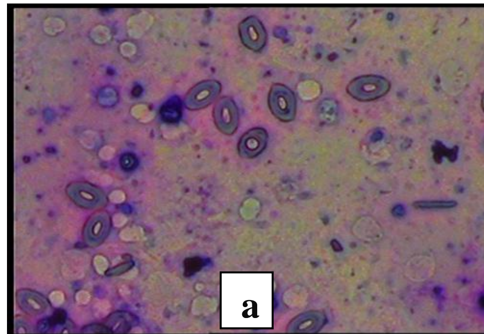
RESULTS AND DISCUSSION

The frequencies of micronuclei in peripheral blood erythrocytes of fish, *Cirrhinus mrigala* induced by the Drug Diclofenac are presented in Figure 1.

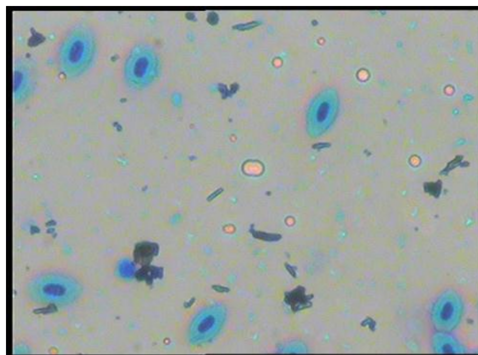
A total of 5000 cells were scored per experimental fishes. The frequency of micronuclei in fishes treated with drug for 24, 48, 72, 96 hours and 10, 20 and 30 days showed significant increase against the control. The frequency of micronuclei ranged between -20% to -480%. Among the different exposure periods, the frequency of micronuclei was higher in 30 days exposure period compared to the control. Different types of micronuclei were observed and was categorized into small micronuclei, large micronuclei, two micronuclei in a cell and notched nuclei.

Cirrhinus mrigala exposed to Diclofenac drug for sublethal concentration to 24, 48, 72 and 96 hours for short term and to 10, 20 and 30 days for long term revealed extensive damage in their micronuclei under pollutant stress. This is an agreement with several earlier observations. Bruno *et al*. (2010) observed the frequencies of MN and nuclear abnormalities

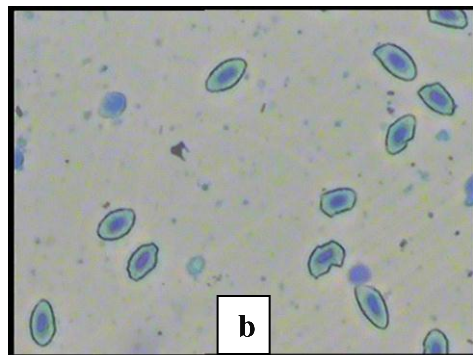
in peripheral fish erythrocytes from fish showed a significant increase in ENA's frequency. Lopez Gonzalez *et al*. (2013) studied on the induction of micronuclei in broad snouted caiman, *Caiman latirostris* hatchlings exposed in vivo to roundup (glyphosate) concentrations used in agriculture.



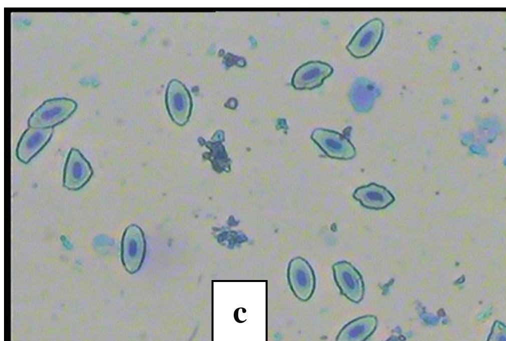
Control



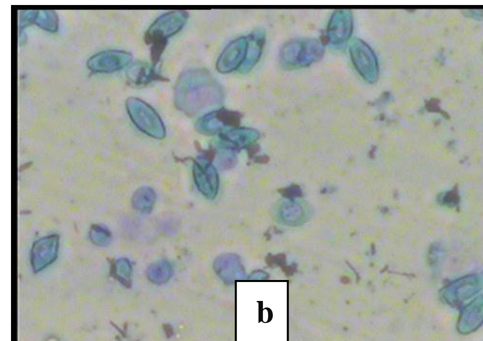
24 hours



48 hours



72 hours



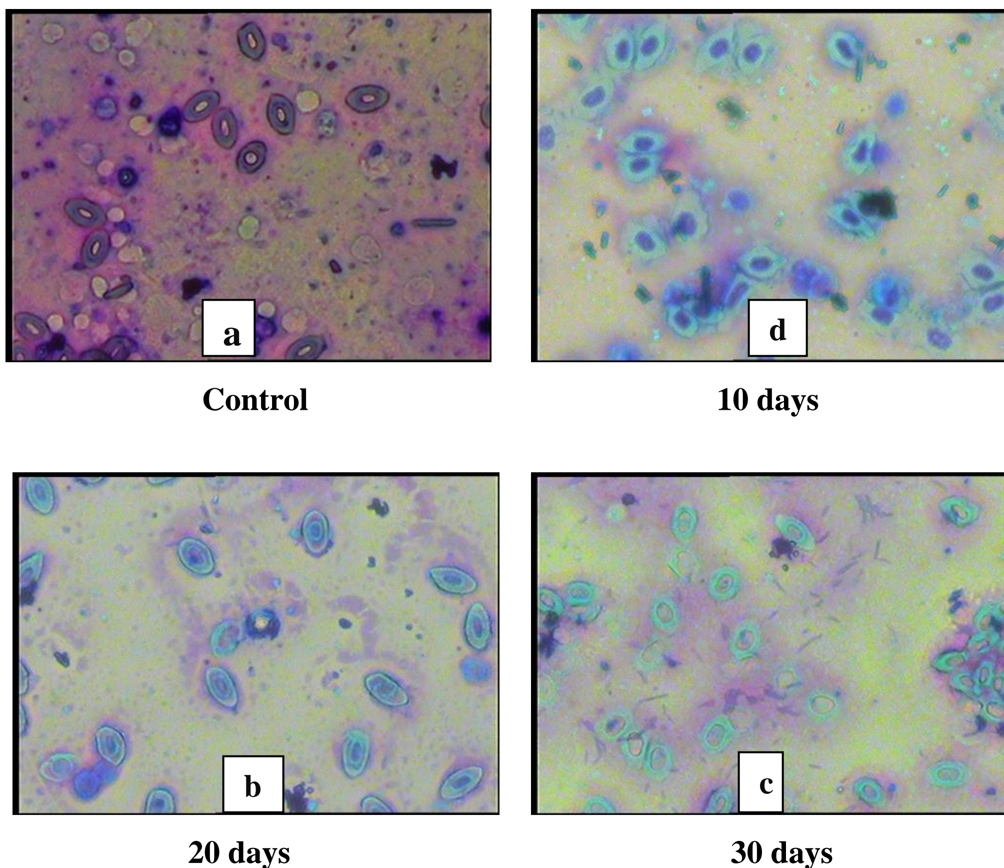
96 hours

a.Small micronucleus

b. Large micronucleus

c. Two micronuclei in a cell

Plate – I: Micronucleated peripheral blood erythrocytes (MN) of *Cirrhinus mrigala* on exposure to drug Diclofenac at short term exposure periods



a. Small micronucleus

b. Large micronucleus

c. Two micronuclei in a cell

d. Notched nuclei

Plate – II: Micronucleated peripheral blood erythrocytes (MN) of *Cirrhinus mrigala* on exposure to drug Diclofenac at long term exposure periods

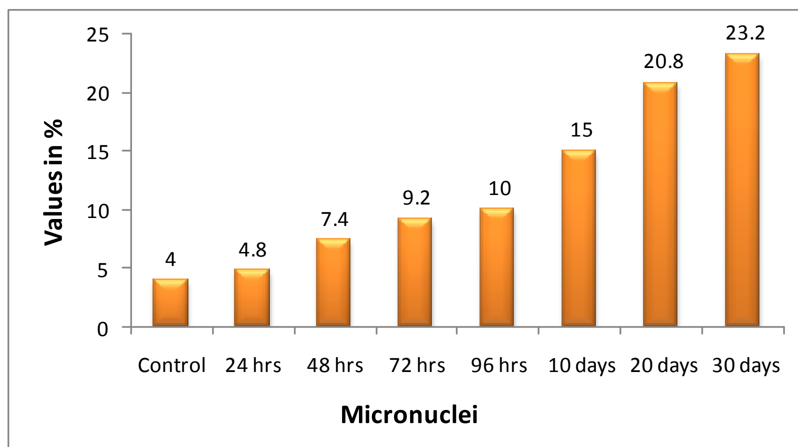


Figure.1. Incidence of micronuclei in peripheral blood erythrocytes of *Cirrhinus mrigala* exposed to Drug Diclofenac at different exposure periods

REFERENCE

1. Halling-Sorensen, B., Nors Nielsen,S., Lanzky, P.F., Ingerslev,F., Holten Lutzhoft, H.C. and Jorgensen, S.E. Occurrence, fate and effects of pharmaceutical substances in the environment. Chemosphere., 1998; 36: 357-393.
2. Lemos, C.T. and Erdtmann, B. Cytogenetic evaluation of aquatic genotoxicity in human cultured lymphocytes. Mutation Research. 2000; 467(1): 1-9.
3. Pacheco, M. and Santos, M.A. Biotransformation, genotoxic and histopathological effects of environmental contaminants in European eel (Anguilla Anguilla L.). Ecotoxicology and Environmental Safety. 2002; 53: 331-347.
4. Lemos, C.T., Rodel, P.M., Terra, N.R., Oliveira, N.C.A. and Erdtmann, B. River water genotoxicity evaluation using micronucleus assay in fish erythrocytes. Ecotoxicol. Environ. Saf., 2007; 66: 391-401.
5. Yadhav, K.K. and Trivedi, S.P. Sublethal exposure of heavy metals induces micronuclei in fish, Channa punctata. Chemosphere. 2006; 77: 1495-1500.
6. APHA, 2005. Standards methods for the examination of water and waste water. 21st Ed. Washington DC.
7. Anon, 1975. Recommendations of the committee on methods for toxicity tests with fish, macro-invertebrates and amphibians. EPA, Oregon. 61.
8. Jack de Brujin, Eddy yedema, Willen senior and Joop Heimeus. Lethal body burdens of four Organophosphorous pesticides in the guppy (Poecilia reticulata). Aquatic Toxicology. 1991; 20: 111-122.
9. Finney, D.J. 1971. Probit analysis, Cambridge University press, London. 333.
10. Schmid, The micronucleus test. Mutation. Res., 1975; 31: 9-15
11. Bruno A.Galindo., Gabriel Troilo, Ilce Mara S Colus, Claudia B.R.Martinez, Silvia H. Sofia. Genotoxic effects of aluminium on the Neotropical fish Prochilodus lineatus. Water Air Soil Pollut., 2010; 212: 419-428.
12. Lopez Gonzalez, E.C., Latorre, M.A., Larriera, A.P., Siroski, A. and Poletta, G.L. Induction of micronuclei in broad snouted caimen (Caimen latirostris) hatchlings exposed in vivo to roundup (glyposate) concentrations used in agriculture. Pesticide Biochemistry and Physiology. 2013; 105: 131-134.