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Genotoxic Effects of Chlordecone in the Cichlid Fish, *Etroplus Maculatus* (Bloch, 1795) using Micronucleus Test

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Abstract

Chlordecone is an organochloride insecticide mainly used for the control of agricultural pests. The genotoxicity of chlordecone was evaluated through micronucleus test in peripheral erythrocytes of Etroplus maculatus. Chlordecone at sublethal concentration (3.5µg/ l) was exposed to fish for 24, 72 and 96 h durations along with the control groups. The results showed that in vivo chlordecone exposure significantly increase the incidence of micronucleus formation in erythrocytes of fish. In the control groups, no nuclear abnormalities were observed, whereas in chlordecone-exposed groups showed nuclear aberrations such as formation of micronucleus together with blebbed, notched, lobed and irregular nuclei. The changes were found to be positively correlated to the duration of exposure. The present data clearly demonstrated that chlordecone is genotoxic at sublethal concentration in cichlid fish, Etroplus maculatus.

Keywords: Chlordecone, Genotoxicity, Micronucleus, *Etroplus maculatus.*

Introduction

Pesticides are omnipresent in the environment and they are extensively applied for the control of a wide diversity of agricultural and household pests that are harmful to the crops and livestock for enhancing the productivity. Instead of having beneficial role in agricultural fields, pesticides can also be hazardous to humans, other animals and the ecosystems due to the contamination in food, water, soil and air. Chlordecone is one of the chlorinated, polycyclic, ketonic pesticides which have been used substantially for the control of leaf eating insects, fire ants, slugs, snails and cockroaches. Chlordecone accords a crucial threat for aquatic ecosystems because of its stability and persistence in sediments, its bioaccumulation in food chains, and its acute and chronic toxicity.

Earlier observations and toxicological studies have demonstrated that chlordecone elicits a number of toxic effects including oxidative stress, histopathological alterations, behavioural changes, immunological alterations, genotoxicity, endocrine disruption, reproductive and developmental toxicity in different organisms[1-3]. Chlordecone has a high potential for bioaccumulation in fish and other aquatic organisms and is resistant to degradation in the environment[4].

Persistence of chlordecone in the aquatic ecosystem is of great concern due to the potential adverse effects on non-target population such as fish. Fishes are the excellent laboratory model for the study of genotoxic potential of contaminants in aquatic ecosystem since they can metabolize, concentrate and store water-borne pollutants[5]. Micronucleus test, one of the most popular tests of environmental genotoxicity is widely used for the evaluation of cytogenetic damage[6]. It is one of the simplest, consistent, inexpensive and rapid screening method for studying both clastogenic (Chromosomal breakage) and aneugenic (spindle fibre dysfunction) effects[7]. Micronuclei are cytoplasmic chromatin masses with the appearance of small nuclei that arise from chromosome fragments or from intact whole chromosomes lagging behind in the anaphase stage of cell division. The presence of micronucleus in cells is a reflection of structural and/or numerical chromosomal aberrations arising during mitosis[8].

There are several studies on the toxic effects of chlordecone on fish; however, data regarding genotoxic potential of chlordecone in fish is rare. Therefore, in the present study *Etroplus maculatus* was used as a model organism to investigate the genotoxic effects of chlordecone using micronucleus test *in vivo*.

Materials and Methods

Animal: The Cichlid fish, *Etroplus maculatus* weighing 7 ± 0.5 g and length 7 ± 1.5 cm were collected from local fish farm near Parappanangadi, Malappuram district, Kerala, India. Fishes were acclimatized to the laboratory conditions prior to experiments and were maintained in aquarium tanks (40 L capacity), which was dechlorinated and sustained with good aeration.

Preliminary tests: The physico-chemical features of the tap water were estimated as per APHA [9]. Water temperature in the test ranged from 28 ± 2°C during the experiment, oxygen saturation of water ranged between 70 and 100 %, pH is 6.5 to 7.5 which were monitored using a standardized procedures.

Chemicals: Technical grade organochloride insecticide, chlordecone (Kepone, decachlorooctahydro-1,3,4-metheno-2H-cyclobuta[cd]-pentalen-2-one, 99.9% purity) was obtained from Supelco, USA. Fetal calf serum, methanol and Giemsa stain were obtained from Himedia Laboratories, Mumbai, India.

Treatments: Chlordecone was dissolved in 1% DMSO, therefore it is used as a vehicle in the experiment. The median lethal concentration value for 96h (LC_{50} – 96 h) of chlordecone was determined as 35 µg/ L[10] by probit analysis. Based on the LC_{50} – 96 h value, a single sub-lethal concentration ($1/10^{th}$ of LC_{50}) with three durations was selected in the present study. Ten animals were maintained in each group.

Group I: Solvent-free control, Group II: Vehicle (1% DMSO), Group III: Chlordecone at 3.5 µg/ L for 24 h, Group IV: Chlordecone at 3.5 µg/ L for 72 h, Group V: Chlordecone at 3.5 µg/ L for 96 h.

Killing of animals: At the end of every treatment period, fishes were caught very gently using a small dip net, one at a time with least disturbance. A drop of blood was collected by cardiac puncture and used for micronucleus test.

In Vivo Micronucleus test: The micronucleus test was performed according to Heddle J.A.[11] and Schmid W.[12] and nuclear abnormalities were evaluated according to Carrasco K.R., Tilbury K.L. and Myers M.S.[13] with slight modification. A drop of blood from fish was taken by cardiac puncture and mixed well with a drop of fetal calf serum in a clean glass slide and smeared. It was then air dried followed by fixing in absolute methyl alcohol for ten minutes. The slide was then stained with 5% Giemsa for 10 minutes and 1000 cells were scored for nuclear abnormalities that were observed under microscope at 100x magnifications. MN frequency was calculated using the formula:

MN % = Number of cells containing micronucleus /Total number of cells counted × 100

Statistical analyses: Statistical analysis was performed using Students t-test by statistical package SPSS 19.0. Differences were considered to be significant at $p<0.05$ against control group. Data are presented as mean ± SD for ten animals per group and experiments were carried out in triplicate.

Results and Discussion

Chlordecone exposure showed formation of micronucleus along with other nuclear abnormalities as blebbed, notched, lobed and irregular nuclei at varying percentage in time-dependent manner as shown in Table- 1 and the morphological abnormalities were shown in Figures 1- 4.

Discussion: Several toxic pollutants that are released daily into the environment, some of natural origin and others due to human activity, pose threat to the dwelling organisms in the environment. Aquatic ecosystem is considered to be more polluted, as it receives many anthropogenic contaminants leached from industrial and agricultural wastes dumped untreated into the water surface. Chlordecone, one of the known persistent organic pollutants, undergoes no significant biotic or abiotic degradation in the environment and are seen persistent in soils and aquatic environment where it get released. Chlordecone alone or in combination with carbon tetrachloride has been experimentally proved as genotoxic agent using the *in vivo* and *in vitro* animal model[14].

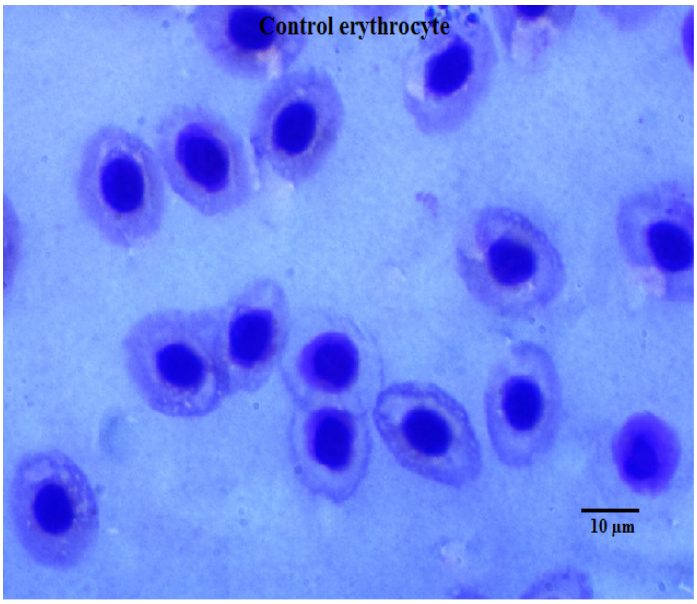

Figure-1
Erythrocyte of control fish showing normal RBC

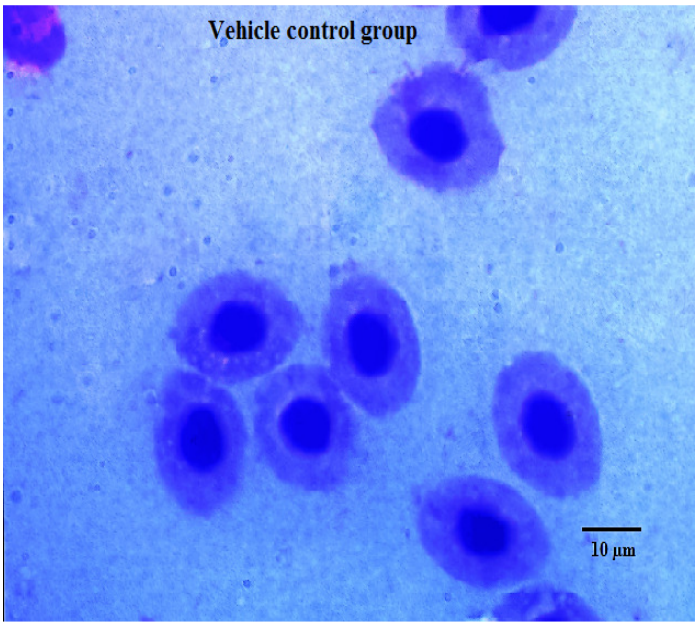

Figure-2

Erythrocyte of vehicle (DMSO-treated) fish showing normal RBC

Genotoxicity is a detrimental action, which affects the genetic material of a cell, thereby affecting its integrity[15]. There are several genotoxic substances, which are known to cause mutagenicity and carcinogenicity, through genetic mutation leading to development of human tumors or cancers[16]. Hence, the present study is an attempt to evaluate the genotoxic potential of chlordecone at sub-lethal concentration in the erythrocytes of cichlid fish, *Etroplus maculatus.*

Fish have been used widely as an experimental model in cytogenetic analysis, since they are relatively inexpensive, simple to handle and retain in the laboratory. Fish erythrocytes have been widely used in genotoxicity as it brings rapid response without much suffering on the part of the organisms and also avoids the complex preparation of cells[17]. Micronucleus test is an ideal genotoxic monitoring technique that uses aquatic organisms to assess the genotoxicity of pollutants in water. This test is applicable for evaluating the clastogenic (that break chromosomes) and aneugenic agents (that induce aneuplody or abnormal chromosomal segregation) in *in vivo* and *in vitro* experiments[5].

The present observation clearly provides convincing evidence that the erythrocyte micronucleus assay is a valuable tool for monitoring genotoxic agents in aquatic ecosystem. In the present study, acute exposure to chlordecone at sublethal concentration significantly increased the frequency of micronuclei formation in time-dependent manner. Other nuclear abnormalities as blebbed, notched, lobed and irregular nuclei were also observed were the frequency increased with increasing durations as compared with the control groups. The results of present study with several nuclear abnormalities demonstrate the genotoxic effect of chlordecone in cichlid fish at controlled laboratory conditions; however, the same parameters are necessary to be confirmed through field experiments.

Table-1
Effect of chlordecone (3.5 µg/ L) on micronuclei and other nuclear abnormalities in erythrocytes of *Etroplus maculatus*

Treatment groups	Micronucleus	Blebbed nucleus	Notched nucleus	Lobed nucleus	Irregular nucleus
Control	-	-	-	-	1.77±0.32
DMSO	-	1.03±0.06	-	-	1.43±0.40
Chlordecone -24 h	8.33±0.42	4.1±0.2	1.87±0.21	2.43±0.40	3.37±0.47
Chlordecone -72 h	24.33±0.61*	8.33±0.45*	7.1±0.7*	10.17±0.40*	7.4±0.5*
Chlordecone -96 h	31.63±1.03*	12±0.9*	13.33±0.83*	18.6±0.53*	11.5±0.5*

Values expressed in Mean ± SD; * denotes significance at p<0.05 against controls.

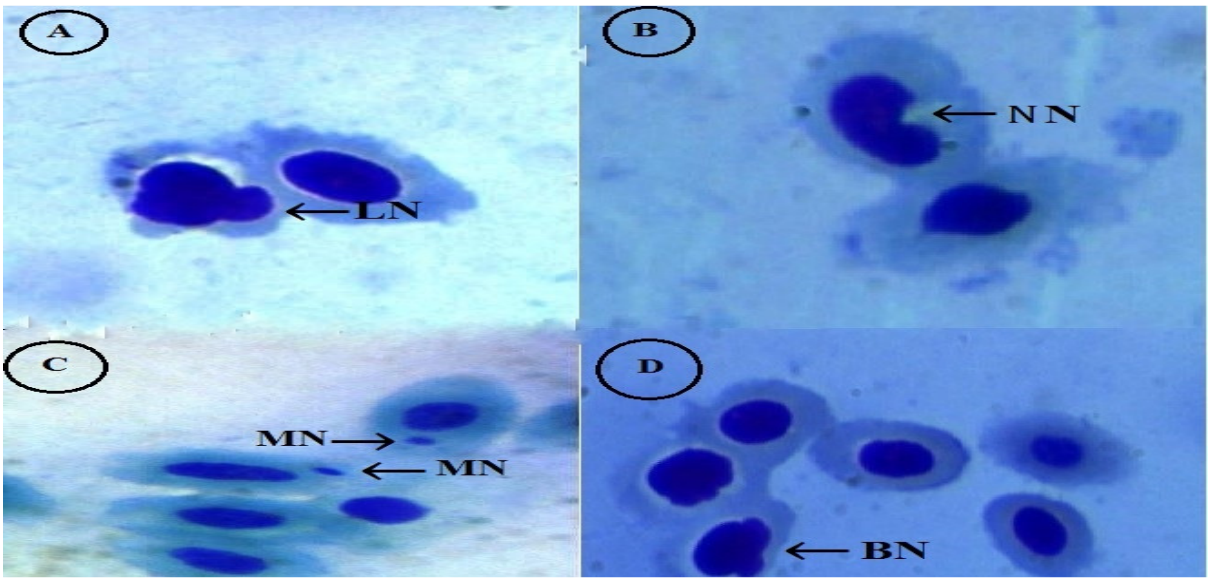

Figure-3

Chlordecone treated fish showing erythrocytes with lobed nucleus (LN), micronucleus (MN), notched nucleus (NN) and blebbed nucleus (BN)

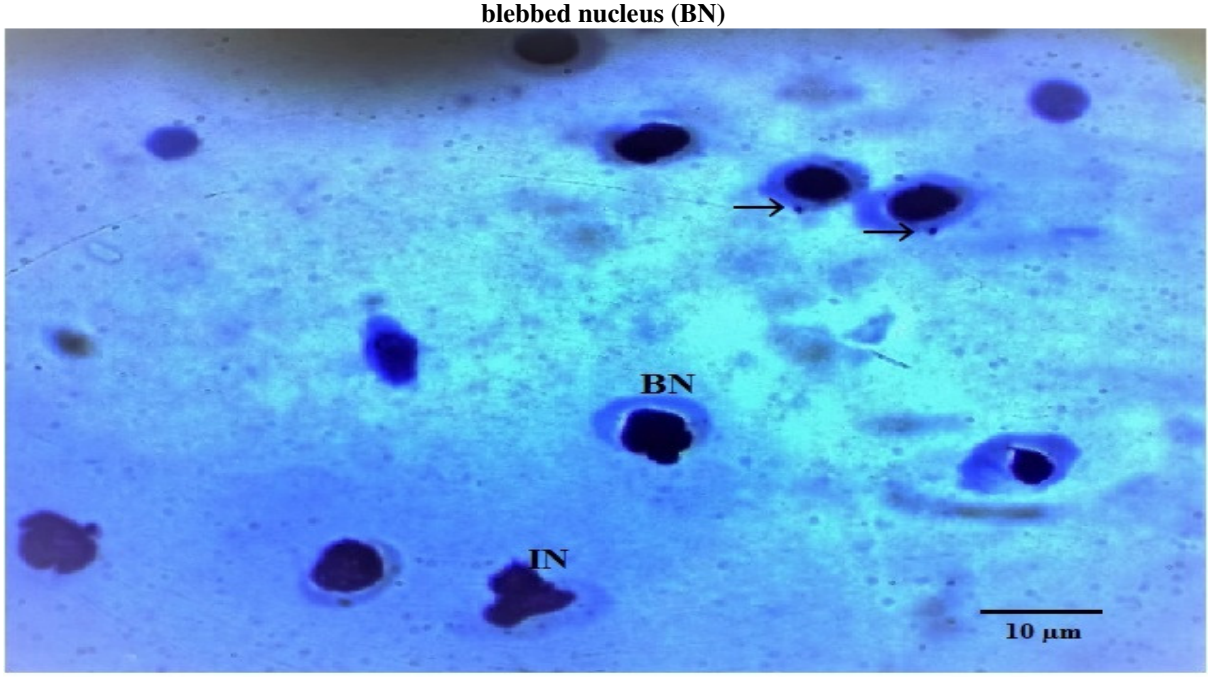

Figure-4
Chlordecone treated fish showing erythrocytes with micronucleus (→), irregular nucleus (IN) and blebbed nucleus (BN)

Conclusion

To brief, micronucleus frequency in fish erythrocytes provides a useful index of accumulated genetic damage due to acute chlordecone exposure at sublethal concentration and also provides an index of response of fish to the contaminant that contributes to the genotoxic impact.

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