International Journal of Research in BioSciences
Vol. 1 Issue 2, pp. (32-37), Oct 2012
Available online at http://www.ijrbs.in
ISSN 2319-2844

Research Paper

Micronucleus assay: a sensitive indicator for aquatic pollution

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(Received 02 July, 2012, Accepted 20 September, 2012)

Abstract

The aquatic resource is the major part of our environment, therefore its safety is directly related to our health. In this study, fresh water fish *Channa punctatus* was taken as a test model to estimate water pollution using micronucleus (MN) assay. The test has been used successfully as a mutagenic assay. The fish was exposed in vivo to three different concentrations (MC, MC/2 and MC/5) of two pesticides (Chlorpyriphos and Malathion) at different time periods (5,10,15,20,25days).Peripheral blood samples smears were stained with Giemsa , MN frequencies were counted and statistically analysed. Result from this study recommends the use of the micronucleus test in fish erythrocyte as a sensitive indicator for evaluation and assessment of aquatic pollution.

Keywords: Fish, *Channa punctatus* Micronuclei Genotoxic, Cytotoxic, Pesticides, Chlorpyriphos, Malathion.

Introduction

Various industrial and agricultural activities increase pollution, particularly in the aquatic environment, which is contaminated by various toxic chemicals from the discharge of waste waters and agricultural drainag [1]. These are responsible for multiple effects at the organisms, including humans, affecting organ function, reproductive status, species survival, population size and ultimately biodiversity. Among these, carcinogenic and mutagenic compounds are the most problematic as their effect may exert a damage beyond that of individual and may be active through following generations. Epizootic neoplasm has been found in a variety of ectothermic species, such as shell fish, echinoderms, jawless fish and bony fish.

Fish are excellent subjects for the study of the mutagenic and/or carcinogenic potential of contaminants present in water samples since they can metabolize, concentrate and store water borne pollutants[2]. Since fish often respond to toxicants in a similar way to higher vertebrates, they can be used to screen for chemicals that are potentially teratogenic and carcinogenic in humans. The main application of fish using as a test model is to determine the distribution and effects of chemical contaminants in the aquatic environment [3] evaluated monitoring systems that use aquatic organisms to assess the genotoxicity of water in the field and in the laboratory. Micronucleus assay was shown to be applicable to fresh water and marine fishes and that gill cells are more sensitive than hematopoietic cells to micronucleus inducing agents The micronucleus test, developed by [5],is an in vivo and in vitro short-time screening method is widely used to detect genotoxic effects . It is one of the simplest, reliable, least expensive and rapid

screening system for both clastogenic(chromosome breakage and formation of acentric fragments) and aneugenic (chromosome lagging and effects on spindle) effects [7]. Clastogenic and aneugenic agents are known to affect the spindle apparatus, and can be differentiated on the basis of the relatively induced micronucleus sizes or in the presence of kinetochores [8]. The micronucleus (MN) test, one of the most popular tests of environmental genotoxicity has served as an index of cytogenetic damage [9, 10].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 stageof cell division. Their presence in cells is a reflection of structural and/or numerical chromosomal aberrations arising during mitosis. [6, 11]

The formation of morphological nuclear abnormalities (NAs) was first described in fish erythrocytes by [12] NAs, including lobbed (LB), blebbed (BL), and notched (NT) nuclei and bi nucleated (BN) cells, have been used by several authors as possible indicators of genotoxicity . Several studies have shown that erythrocytes of fish present a high frequency of micronuclei and nuclear abnormalities after exposure to different heavy metals under both fi eld and laboratory conditions [1, 3, 13]

For the determination of genotoxic effect in fish, the micronucleus test as well as the study of the abnormal shape of nuclei is a suitable measure with which the presence or absence of genotoxins can be detected in water. The detection of MN and NAs in fi sh help us to assess the status of water quality as well as the health of a particular species and any potential risk it might have after consumption [14].

Since micronucleated piscine erythrocytes have been proved to be sensitive indicators of genetic damage, the purpose of our study was to evaluate the cytogenetic (clastogenic or aneugenic) effects of Malathion and Chlorpyriphos in *Channa punctatus* fish using the MN test [15-17].

Materials and Methods

Test animal: Specimens of *Channa punctatus* measuring about 10-12 cm collected from the local ponds and maintained in laboratory aquaria were acclimatized for a fortnight before treatment.

Test Chemical : Chloropyriphos (0,0-diethyl-0-(3,5,6 trichloro-2 pyridinyl)thio sulphate)and Malathion S-[1-2-bis(ethoxycarbonyl) ethyl,0-0dimethyl] are the organophosphorous insecticides bought from the local market.

Doses and route of exposure: From among the specimens acclimatized for at least a fortnight in the laboratory aquaria, only strong and active fishes were released into different aquaria containing 250µ/L, 125µ/L,67.5/L. µand 25 µ/L of Malathion and 10 µ/L,5 µ/L,2.5µ/L and 1µ/L of Chloropyriphos which correspond to $LC_{50,}$MC, MC/2 and MC/s doses respectively. MC represent the maximum tolerable concentration of the test compound at which no death of animal beyond 5% was observed during the period of treatment and was determined from preliminary experiments on groups of 20 specimens in aquaria containing 100 litre of water. The test lasted for 25 days with change of water, chemical and food every alternate day. The lowest concentration leading to 50 % death after the treatment was considered as LC_{50} and half of this corresponds of MC. MC/2 and MC/5 represent 1/2 and 1/5 of MC. The treated specimens received an intramuscular injection of 0.02% colchicine solution at the rate of 1ml / 100 mg body weight 2 h prior to their completion of 5,10,15,20 and 25 days of exposure to the test chemical.

Micronucleus Test: The smear of peripheral blood drawn from the caudal vein with a heparinized syringe, was prepared and well-dried slides were stained in 10% Giemsa solution (Stock solution diluted with Sorensen's buffer at pH 6.8) for 30 min following the method of [5]. Four thousand cells per animals (1000 cells per slide) were scored for micro-nuclei and nuclear anomalies.

Statistical analyses: For comparison of mean ,Standard deviation, Student's t-test was applied. Two-way analysis of multiple variances (ANOVA) was done for dose and time response

Results

Micronuclei(MN) induced by Malathion and Chlopyriphos in the peripheral erythrocytes were generally dot shaped and were close to the main nucleus with size and shape veries among cells. Mostly each affected erythrocyte had a single MN while few cells contained more than one micronucleus. A few erythrocytes exhibited nuclear anomalies like blebbed, notched, vacuolated nuclei etc. The frequency of micronuclei and nuclear anomalies of various kinds in the peripheral erythrocytes in *Channa punctatus* exposed to different concentrations of Chlorpyriphos and Malathion for varying periods of time and in control is summarized in table 01 and 02 respectively. Evidently, all the treated group of specimens had higher frequency of erythrocytes with MN and nuclear anomalies as compared to controls. The frequency of micronuclei in peripheral erythrocyte increased progressively with the increase in the period of exposure and/or concentration of the Chlorpyriphos.

Statistical analysis of the data revealed that the increase in the frequency in treated specimens was not only significant as compared to the control but also the level of significance increase with the increase in the concentration or period of exposoure, thereby indicating that chlorpyriphos induced micronuclei in dose and period dependent manner. Two way analysis of multiple variance(ANOVA test) also testified the dose and period dependent increase in frequency in treated groups of specimens. In fact the calculated values of 'F' for concentration ($F=158.20$, $d.f.=14.2$ $p\leq0.001$) and period of exposure($F=335.95$, $d.f.=14.4$ $p\leq0.001$)were significantly higher than their tabulated values.

Malathion treated group of specimens had also significantly higher frequency of MN as compared to control to controls (Table 2).The frequency increased with increased in concentration till 20 days. The specimens exposed for 25 days, in the other hand had slightly a lower frequency than those exposed for 20 days to any of the three concentrations, as was the case with most organ phosphorous pesticides described earlier. Statistical analyses of the data, however, revealed that the increase in the frequency in all specimens was statistically significant as compared to the controls and that the level of significance increased with increase in the concentration or period of exposure. Two-way analyses multiple variance also revealed that the compound induced MN and nuclear anomalies in concentration as well as period of exposure dependent manner as was the case with all pesticides studies herein. In fact, the calculated values of 'F' for concentration ($F133.88$, $d.f.14.2$, $p<0.001$) and period of exposure ($F=89.11.df$ 14, 4, $p<0.001$) were significantly higher than their respective tabulated values.

Table 1: Frequency of peripheral erythrocyte with micronuclei and nuclear anomalies in peripheral erythrocytes of *Channa punctatus* after *in vivo* exposure to 3 different concentrations of Chlorpyriphus for 5-25 days and control

Periods of Exposure (in days)	Concentrations MC/5	MC/2	MC
Control	0.055 ± 0.007	0.055 ± 0.007	0.055 ± 0.007
5	0.330±0.023	0.416±0.039	0.499±0.036
10	0.429±0.042	0.525±0.033	0.609±0.060
15	0.565±0.059	0.659±0.041	0.722±0.069
20	0.659±0.043	0.743±0.074	0.845±0.093
25	0.722±0.063	0.872±0.085	0.947±0.093

Table 2: Frequency of peripheral erythrocyte with micronuclei and nuclear anomalies in peripheral erythrocytes of *Channa punctatus* after in vivo exposure to 3 different concentrations of Malathion for 5-25 days and control

Periods of Exposure (in days)	Concentrations MC/5	MC/2	MC
Control	0.055 ± 0.007	0.055 ± 0.007	0.055 ± 0.007
5	0.101±0.018	0.302±0.029	0.351±0.026
10	0.243±0.019	0.374±0.028	0.451±0.039
15	0.333±0.025	0.442±0.041	0.543±0.039
20	0.431±0.038	0.542±0.034	0.633±0.051
25	0.394±0.026	0.493±0.052	0.592±0.046

Discussion

Micronucleus bioassay offers several types of unique information as a simple bioindicator for chromosomal aberrations not available from other methods: (1) the integrated effect of a variety of environmental stresses on the health of an organism and the population, community, and ecosystem (2) early warning of potential harm to human health based on the responses of wildlife to pollution, and (3) the effectiveness of remediation efforts in decontaminating waterways [18]. In fish, the micronucleus test is usually based on erythrocytes, but liver and gill tissues have been used [19] compared between the micronucleus frequencies of kidney and gill erythrocytes in tilapia fish, following mitomycin C treatment detecting no significant difference between the frequencies of the micronuclei. Similarly, Manna and [20] maintained that there was no statistically significant difference between the frequency of micronuclei in gill and kidney cells after irradiation in the two tissues. While they included various types of cells, our study was focused on the erythrocytes. A hypothesis to explain the fact that we did not detect any difference between kidney and peripheral blood micronuclei counts may be that circulating peripheral erythrocytes also undergo mitosis [19].

However, if the kidney is the main hemopoietic tissue in fish, and if micronuclei are formed during cell proliferation [19, 22] more micronucleated erythrocytes should be expected in the kidney than in the gill. Alternatively, as reported by [19] we may have sampled peripheral blood during kidney imprinting and practically, the cephalic kidney is a frequently chosen organ for cytogenetic aberration studies in fish. There was no significant difference (P> 0.05) between the frequencies of micronuclei obtained in *Synodontis clarias* and *Tilapia nilotica*[23] . observed the African walking catfish, *Clarias gariepinus* from freshwaters of Egypt to have higher incidence of the chromosomal aberrations of micronuclei in its genome than the three tilapia species employed in the study, maintaining the species to be highly tolerant of that particular genetic damage without triggering the genetically programmed event that allows cells to commit suicide [23] In our present study,it has been find out that Chlorpyriphos is comparatively more toxic than Malthion both period and dose of exposure[15-17] .

Conclusion

The results demonstrated that different fish species can respond in completely different ways to a given genotoxic agent. Depending on the toxic agent and on the species, the behavior of micronuclei rates may exhibit significant variations, probably related to the chemical kinetics of the toxins and to the speed of the hemopoietic cycle [24]. It is recommended that micronuclei tests in fish erythrocytes be carried out at various times, thus making it possible to follow-up the changing micronuclei frequencies. The sampling of the peripheral blood is appropriate since it allows collecting several samples from the same individuals, without having to sacrifice it [25]. So employing Micronucleus test for qualitative analysis of water useful as a sensitive indicator for cytogenetic study

Acknowledgement

Author is thank full to Prof. R. Prasad, HODs of PG department of Zoology, Berhampur University and Principal, Indira Memorial College, Chandiput ,Gajapati, Odisha.

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