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STUDIES ON THE TOXICOGENOMIC EFFECTS OF ORGANOPHOSPHATE PESTICIDE DIMETHOATE [O,O-DIMETHYL S-(N-METHYLCARBAMOYLMETHYL) PHOSPHORODITHIOATE] IN *CYPRINUS CARPIO* L.

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ABSTRACT

Aim: Since 1940s, chemical pesticides of one form or another have become a dominant and essential form of pest control throughout much of the world. Because of the widespread use of pesticides for domestic and industrial applications, the evaluation of their toxic effects is of major concern to public health. The aim of the present study was to investigate the toxicogenomics effects of dimethoate (DM), an organophosphorus pesticide, to target the chromosomes of fish.

Methodology: In the present investigation, two species of *Cyprinuscarpio* L. i.e. *Cyprinuscarpiospecularis* and *Cyprinuscarpiocommunis* were treated with three sub-lethal concentrations of DM (5 ppm, 10 ppm, & 15 ppm.). After treatment, the frequency of micronuclei was examined at the duration of 24, 48 and 72h. The micronucleus test (MNT) was used for assessing the genotoxicity of the fishes by the preparation of slides and scoring of micronuclei.

Results: The pesticide treatment caused significant changes in the frequencies of micronuclei in erythrocytes of *Cyprinuscarpiospecularis* and *Cyprinuscarpiocommunis.* Micronuclei were non-refractive small nuclei lying near the main nucleus displaying the same pattern as the main nucleus. Nuclear abnormalities were like cells with binuclei, blebbed nuclei, lobed nuclei and notched nuclei.

Conclusion: It was concluded that the pesticide dimethoate possess mutagenic potential at varying extent. A time and dose dependent increase in the frequency of micro-nucleated erythrocytes was found.

Keywords: Toxicogenomics, dimethoate, micronucleus, *Cyprinuscarpio*

INTRODUCTION

At present, the pesticide manual includes 3,100 main entries and list over 10,400 products (1-3). Many of them are suspected to have mutagenic and carcinogenic activities (4). Organophosphate pesticides are finding increasing use in recent years since they are biodegradable and therefore persist in the environment only for a short time. Because of their low persistence, repeated applications of these pesticides for the control of pests in agricultural fields and thereby large quantities find way into water bodies (5, 6). Their extensive application may affect fish population as they enter the water through irrigation or rain. The organophosphate compounds are esterase inhibitor of neurotoxicants (7) with acute cholinergic effect preceded by inhibition of acetyl cholinesterase (8). Being neurotoxicants, organophosphate compounds interfere with many of the vital physiological functions (9, 10) and consequently alter the levels of various body constituents (11)

Though the application of pesticides is based on their toxicity to selective pests, it is not specific, resulting in very hazardous effect, particularly on aquatic organisms since pesticides eventually reach aquatic ecosystems in considerable amounts as agricultural run-off and outputs from municipal water treatment and manufacturing plants. Many contaminants present in aquatic environment not only endanger the survival and physiology of the organisms but also induce genetic alterations, which may lead to mutation and cancer (12-16). DM, most widely used insecticide, is a particular concern to those exposed occupationally during manufacture, formulation and use. DM exerts toxic effects on many tissues and organs including pancreas (17-20). It is acutely toxic, has possible links to cancer and is suspected of causing birth defects (21). DM was found to be mutagenic in *E. Coli* (22). Although data on acute, subchronic and chronic toxicity of DM in laboratory animals are well documented, it's potential to induce genotoxicity remains unclear. Therefore, the present study is designed to study the genotoxic effects of DM on the two species of fishes i.e. *Cyprinus carpio specularis* and *Cyprinus carpio communis*.

MATERIALS AND METHODS

Chemical Mutagen

The commercial grade of DM was obtained from Premier Sales Agency (Srinagar, India), manufactured from Isagro (Asia) Agrochemicals Pvt. Ltd. (Bathinda, India). CAS Reg. no: 60-51-5, Chemical formula: $C_5H_{12}NO_3PS_2$

Experimental Animal

The present study is carried out by using two species of *Cyprinus carpio* L. (family: Cyprinidae), i.e. *Cyprinus carpio specularis* and *Cyprinus carpio communis*. These fishes were identified by the presence of heavy and strongly serrate spines in the anterior portion of its dorsal and anal fins and by the presence of two rather long, fleshy barbles on each side of its upper jaw (23). The mouth is terminal in the adult and sub terminal in the young (24). *Cyprinus carpio communis* (scale carp) has regular concentric scales and *Cyprinus carpio specularis* (mirror carp) has large scales running along the side of the body in several rows with the rest of the body naked (25). Average age of fishes was below one year, weight was 30-40 g and length was 10-12 cm. After collection of fish specimens were acclimated for 45 days at 28^0C prior to trials. Specimens were kept in polypropylene troughs each with 8-10 individuals/50 L of water. Water was kept O_2 saturated by aeration.

Treatment of DM and selection of the dose

In the present study fishes were divided into two groups i.e. the control and the experimental group. The experimental group for each fish species were divided into three subgroups based on the selected dose of DM. On the basis of the literature data (LC_{50} values for each insecticide), three sub-lethal concentrations: 5, 10 and 15 ppm of DM were selected (26). After treatment with each insecticide, the frequency of micronuclei in all experimental groups were examined at three durations of 24, 48 and 72h. Ten fish specimens were used for at each duration and at each concentration.

Micronucleus Test

The micronucleus test was performed on peripheral blood according to the standard protocols with slight modifications (10, 13, 15, 27)

Slide Preparation

Fishes were killed with a slight blow on the head region. Chemically treated and control fishes were cut in the caudal region and smears of peripheral blood made on grease free clean slides. After fixation the slides were stained with Mayer's haematoxylin, rinsed in Scott's tap water substitute followed by another staining of eosin (13, 28)

Scoring of Micronucleus

For each concentration and duration ten fish specimen were used and from each fish ten slides were studied and 1200 cells were scored under 1000X magnification. Small non-refractive, circular or avoid chromatin bodies, displaying the same staining and focussing pattern as the main nuclei, were scored. Other nuclear abnormalities were also studied and classified as binuclei, blebbed nuclei and notched nuclei (16, 29)

Photomicrography

The slides were carefully studied and various morphological peculiarities of nuclear material were examined under light microscope for accurate scoring of micronuclei. Later photomicrography was conducted with the help of Trinocular microscope (Leica DMLS2) to keep record of all the details observed under microscope.

Statistical analysis

Statistical analysis of data to verify the significant difference in the incidence of micronucleus between treated and control groups at 5% level of significance was performed using non-parametric criteria, Mann-Whitney U test to analyse the frequency of micronuclei. To ensure statistical accuracy, only cells with one micronucleus were considered, while rarely occurred two micronuclei and other nuclear abnormalities were eliminated from the counts.

RESULTS

The pesticide treatment caused significant changes in the frequencies of micronucleus in erythrocytes of *Cyprinus carpio specularis* and *Cyprinus carpio communis.* In these two species of fishes mature erythrocytes are large structures with least differences in size and shape of nucleus. The micronuclei observed were clearly structured with well-defined boundary, which facilitated the identification of fragments in their cytoplasm. Micronuclei were non-refractive small nuclei lying near the main nucleus displaying the same staining pattern as the main nucleus. The number of micronuclei was found restricted to one, or occasionally two, the latter being a very rare phenomenon. Other nuclear abnormalities were also observed like cells with binuclei, blebbed nuclei, lobed nuclei and notched nuclei. But all these nuclear abnormalities were rarely seen and therefore were not scored. The peak frequency of micronucleated peripheral erythrocytes was observed after longer periods of exposure and higher dose treatment. Examples of normal erythrocytes of control fish, micronucleated erythrocytes and other nuclear abnormalities of treated fish are presented (Figure. 1, A-D)

The fishes were exposed to three sub-lethal concentrations of 5 ppm, 10 ppm and 15 ppm. The percentage of single micronuclei in *Cyprinus carpio specularis* (0.03 ± 0.01 of control) increased to 0.32 ± 0.03 from low to high concentrations after 24h and continued to increase by 0.74 ± 0.17 and 1.37 ± 0.16 after 72 h exposure respectively is shown in Table.1. In *Cyprinus carpio communis* the percentage of single micronuclei increased from 0.03 ± 0.01 of control to 0.39 ± 0.06 (24h), 0.76 ± 0.14 (48h) and 2.23 ± 0.49 (72h).

DISCUSSION

The use of fish biomarkers as indices of the effects of pollution are of increasing importance and can permit early detection of aquatic environmental problems (30). The micronucleus test in fish has been applied for both laboratory treatments of in-vivo and in-situ exposure to environmental pollution. Induction of

micronuclei by several well-known clastogenic/mutagenic agents such as cyclophosphamide, mictomycine-C, bleomycine, colchicines, ethyl methane sulphonate and vinblastin was assessed in freshwater and marine fish species (31-33). The efficacy of the micronucleus test as an indicator of cytogenetic damage has already been proven and the studies of micronucleus formation have been successfully used as bioassay to measure the impacts after fish treatment with surface water disinfectants (34), herbicides (12-14, 35, 36) insecticides (37, 38), benzo (x) pyrene and other polyaromatic hydrocarbon compounds (39).

In the present study, positive genotoxic effects, measured as micronucleus frequency in erythrocytes from both fish species (*Cyprinus carpio specularis* and *Cyprinus carpio communis*) exposed to different insecticides were observed. The results of the present study revealed a significant induction of micronuclei in peripheral erythrocytes ($P< 0.01$ and $P<0.05$) of *Cyprinus carpio specularis* and *Cyprinus carpio communis.* The appearance of inter-specific differences observed in the present study could be attributed to the specificity of DNA repair, cell turnover time, physiological peculiarities, contaminant uptake or biotransformation in the fish species studied.

In the present study a significant difference in the micronucleus incidence among treated and control groups was observed. The peak frequency of micronucleated erythrocytes was observed at 72 h after exposure (Figure.2).

A time dependent increase in the incidence of micronuclei in the peripheral erythrocytes of *Cyprinus carpio specularis* and *Cyprinus carpio communis* was established and confirms other observations (31, 34, 40).

Mutagenic effects due to DM exposure were also reported in mice. They were more prominent in male mice given a single high dose of DM than in male mice given one twelfth of the same dose daily for 30 days (41). Several other studies have reported toxic effects of DM on metabolism and enzyme system of various fish species (42- 44).

CONCLUSION

In the present study DM was found to induce micronuclei in fish erythrocytes. Therefore, the present study confirms the genotoxicity of dimethoate in aquatic organisms. Genotoxicity induced by DM could be attributed to the parent compound itself as well as to its oxygen analog omethoate, which was considerably more toxic than DM (45). These effects can also be attributed to active oxygen species generated by DM (17, 46, 47). Thus, further investigations are needed to elucidate the mechanism by which DM induced DNA damage.

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Table 1: Dose dependent incidence of micronuclei %age in dimethoate treated peripheral blood erythrocytes of *Cyprinus carpio specularis* and *Cyprinus carpio communis.*

Treatment	DM Concentration	Fish species	MN frequencies (%)		
			24h	48h	72h
Control	-	*C. p. Specularis*	0.03 ± 0.01	0.05 ± 0.01	0.05 ± 0.01
		C. p. Communis	0.04± 0.01	0.04 ± 0.01	0.05 ± 0.01
Dimethoate	5 ppm	*C. p.* Specularis	0.08 ± 0.02	0.12 ± 0.02	0.22 ± 0.07
		C. p. Communis	0.12 ± 0.05	0.16 ± 0.02	0.27 ± 0.03
	10 ppm	*C. p.* Specularis	0.13 ± 0.02	0.22 ± 0.04	0.37 ± 0.08
		*C.p.*Communis	0.21 ± 0.02	0.28 ± 0.56	0.41 ± 0.04
	15 ppm	*C. p.* Specularis	0.32 ± 0.03	0.74 ± 0.17	1.37 ± 0.16
		C. p. Communis	0.39 ± 0.06	0.76 ± 0.14	2.23 ± 0.49

(Mann – Whitney *U* test) $P < 0.05$

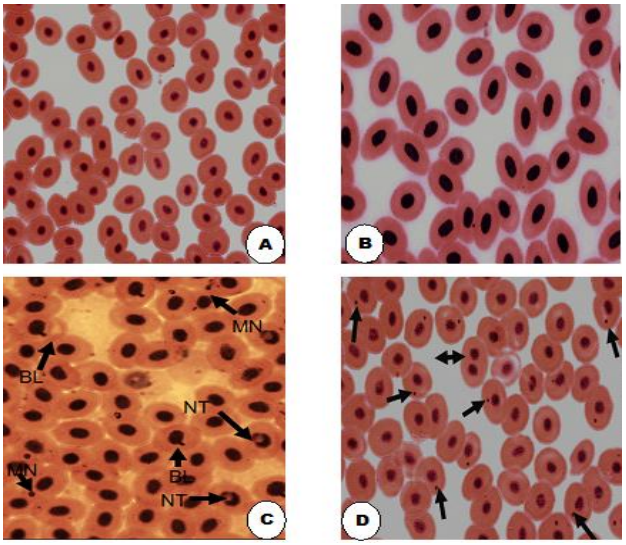

Figure 1

A. Peripheral Erythrocytes of control fish (*Cyprinus carpio specularis*) showing normal nuclear features (1000X).

B. Peripheral Erythrocytes of control fish (*Cyprinus carpio communisis*) showing normal nuclear features (1000X).

C. Micronuclei (MN) and other nuclear abnormalities; BL: blebbed nuclei; NT: notched nuclei in peripheral blood erythrocytes of treated fish, *Cyprinus carpio communis* (1000X).

D. Photomicrographs of treated fish, *Cyprinus carpio specularis* showing variations in micronuclei [arrow] and binuclei [double arrow] (1000X).

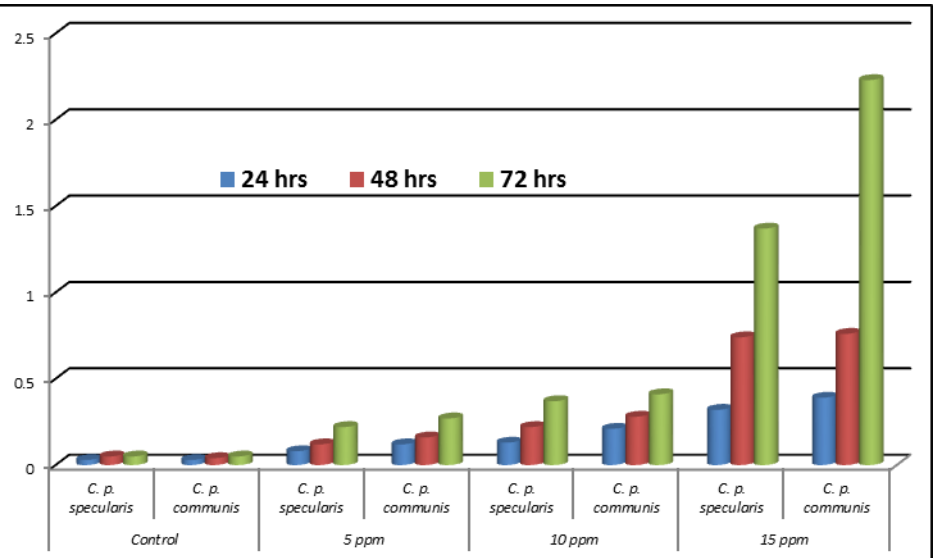

Figure 2: Dose response relationship of micronucleated erythrocytes exposed to dimethoate at different concentrations and durations in *Cyprinus carpio*