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Deltamethrin, a synthetic pyrethroid genotoxic effect as determined by Micronucleus test (MNT) in the blood of *Ctenopharyngodon idella* (Valenciennes)

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ABSTRACT:- *The synthetic pyrethroid of type II with cyanogroup Deltamethrin induced changes in the blood of the fish Ctenopharyngodon idellathat was detected by a simple preliminary experiment as Micronucleus (MNT). The fish are exposed to both lethal and sublethal concentration of 96 hrs (1/10th of LC_{50}) of technical grade and 11% EC Decis Only the fish exposed to lethal concentrations of the technical grade changes in the nucleus whereas in the sublethal concentrations of technical grade and lethal and sublethal concentrations of 11% EC Decis have not resulted any changes. This will pave the way of Carcinogenicity.*

Key words: Deltamethrin, Micronucleus test (MNT), Technical grade, 11% EC Decis

INTRODUCTION

In pesticide Ecological studies, there are different types of markers as indices termed as biomarkers of such, studies are contemplated they are genotoxic studies involving DNA.In pesticide toxicology, the effects are studied for organochlorines, organophosphates, carbamates and synthetic pyrethroid. It is a known fact the contamination of aquatic environment either directly or indirectly is repository and such studies of genotoxicity will help the research paving the way for advanced research. The synthetic pyrethroids, due to low environmental persistence and toxicity, are used instead of organochlorines and other pesticides in pest control management. Ibrahim *et al.* (2014). According to sabzar *et al.*, (2015) due to the contamination of toxicants as residues have genetoxic potential. As the effect may be on DNA resulting 'enduring' and 'ardent consequences'. Hence the genotoxicity is another tool that a physical or chemical agent can exert on the genetic maternal-of an organism including fish. At subtoxic concentration, the outcome produced has to be detected and analyzed. Termed as clastogenic a chemical toxicant in piscine model of Genotoxicology it is a risk factor for genetic diseases in population. The fish have received particular attention as monitor system – for its sustenance and indirectly the human health. A chromosomal aberration tests, Micronucleus assay and comet assay are the ways in laboratory to study such changes at the DNA in the literature. Among the above, the micronucleus is the preliminary test, so study the effect of the pesticides – particularly synthetic pyrethroids. Fish as model, Sabzar *et al.*, (2015) presented, in his review article, have given the list of micronucleus tests to different fishes for different researchers. MNT has become popular tool for assessment genotoxic potential of various chemical agents by using as a fish model. Table 1 some studies on fish for the evaluation of genotoxicity of various xenotic agents using MNT. Hence, in the present study, Deltamethrin a synthetic pyrethroid is tested on the fish *Ctenopharyngodon idella* to study the genotoxicity by a preliminary basic MNT test owing to paucity of laboratory facilities.

MATERIALS AND METHODS

The fresher water grass carp, *Ctenopharyngodon idella* is an edible and economically important fish was selected with a range of size about 3 to 5 cm and 4.5 grams

of weight, irrespective of their sex, have been chosen as the test organisms for present investigation. Health and active fish were obtained from local fish farm. The fish were acclimatized to the laboratory conditions in large plastic water tanks for three weeks at a room temperature of 28±1°C. Water was renewed every day with 12-12 h dark and light cycle. During the period of acclimatization, the fish were fed (*ad libitum*) with groundnut oil cake and rice bran. Feeding was stopped one day prior to the actual toxicity test. All the precautions laid by committee on toxicity tests to aquatic organisms (APHA 2005) were followed and such acclimatized fish only were used for experimentation. If mortality exceeded 5% in any batch of fish during acclimatization, the entire batch of that fish were discarded. The fish were exposed to technical grade 96 h LC_{50} (0.331) and sub lethal 1/10 of 96 hours LC_{50} values (0.0331) and 11% EC 96 h LC_{50} (0.172) and sub lethal 1/10 of 96 hours LC_{50} values (0.0172) for 10 days.

Sample Of Blood

Fish were euthanized by an overdose of MS-222 and then weighed and measured. Blood was sampled by caudal severance from the disease free test fish during the early hours of the day and stabilized with 50 IU sodium heparin (anticoagulant)/ml blood.

Micronucleus (MN) Assay

At each sampling a drop of blood was immediately smeared on a clean slide. On drying, the smears were fixed in methanol for 10 minutes, left to air dry at room temperature and finally stained with 6% Giemsa solution in Sorenson buffer (pH 6.9) for 20 minutes. After drying the slides were rinsed with distilled water to remove extra stain. A total of about 4,000 erythrocytes were examined for each specimen per concentration (2,000 cells per slide) under the light (Olympus) using 100x oil immersion lens binocular microscope.

Table 1. Genotoxic studies as MNT and Comet assays studies on fish

S.No.	Fish	Chemical(s)/Pollutants
1	*Oreochromis mosambicus*	Aldrin, Cadmium chloride and x-rays
2	*Cyprinus carpio and Tinca thinca*	Aflatoxin B1, arochlor 1254, benzidine, benzo(a)pyrene and 20-methylechloanthrene
3	*Heteropneustes fossilis*	Mitocycin C and paper mill effluent: allylformate
4	*Esox lucius*	Radiocesium
5	*Oncorhynchus mykiss*	*In situ* to heavily polluted tributary of the River Po (Northern Italy)
6	*Carassius auratus gibelio*	Selenium, mercury and methyl-mercury
7	*Salmo truttafario*	PCB77
8	*Channa punctatus*	Malathion
9	*Oncorhynchus mykiss*	A textile industry effluent
10	*Cheirodon interruptus interruptus*	Pyrethroid λ-cyhalothrin
11	*Astyanax bimaculatus*	Cyclophosphamide, vinblastine sulfate
12	*Channa punctatus*	Malathion
13	*Oncorhynchus mykiss*	Colchicine, mitomycin, cyclophophamide, acrylamide, methyl-methanesulfonate and N-ethyl-N-nitrosourea
14	*Clarius batrachus*	2,4-dichlorophenoxyacetic acid and butachlor
15	*Heteropneustes fossils*	Pentachlorophenol
16	*Channa punctatus*	Pentachlorophenol and 2,4-dichlorophenoxyacetic acid
17	*Anguilla anguilla, Phoxinus phoxinus and Salmo trutta*	Metals, hydrocarbons, pesticides
18	*Cyprinus carpio*	Disinfectants (sodium hypochlorite, peracetic acid and chloride dioxide)
19	*Cyprinus carpio, Carassius gibelio, Corydoras paleatus*	Cadmium chloride and cooper sulphate
20	*Oreochromis niloticus* and *Tilapia rendalli*	Domestic sewage
21	*Scophthalmus maximus*	Dialkyl phthalate, bisphenol-A, tetrabromodiphenyl ether
22	*Oncorhynchus mykiss*	Mixture of heavy metals
23	*Clarias gariepinus, Oreochromis niloticus and Oreochromis aureus*	Heavy metals
24	*Channa punctatus*	Chlorpyrifos
25	*Canna punctatus*	Malathion
26	*Cnesterodon decemmaculatus*	Aficida (insecticide)
27	*Carassius carassius*	Agricultural runoff
28	*Apteronotus bonapartii*	Benzene
29	*Labro rohita*	λ-cyhalothrin
30	*Carassius carassius*	Endosulfan

The characteristics used for the identification of the micronucleus were circular or oval bodies having no connection with the main nucleus, smaller than one-third of the main nucleus and showing the same staining and focusing pattern as the main nucleus. Micronucleus frequency was calculated from the formula:

$$MN\% = \frac{\text{Number of cells containing Micronucleus}}{\text{Total number of cells counted}} \times 100$$

Results And Discussion

The results of micronuclei for 1000 blood cells of *Ctenopharyngodon idella* is given in table 2, and image of stained micronucleus in Figure 1. A control of 1000 erythrocyte blood cells showed changes and the image has given indication of toxic action as micronucleus a clastogenic nature – only in lethal concentration of Technical grade and 11% EC of Deltamethrin. No changes are observed in sublethal concentration exposure of both TG and 11% EC.

Gadhave *et al.*, (2014) reported on λ-cyhahalothrin induced genotoxicity in freshwater fish *Labeo rohita*. *Labeo rohita*, constitute 35% of production among major carps as there were no gentoxic studies attempted and the findings with the type I synthetic pyrethroid, as a toxicant is that frequency of micronuclei increased with the concentration and deceased with time. The geno-toxic compound cyalophasmide a known genotypic acts as alkylating agent which have the power of cleaving the genetic material. The present study even though the one exposed to synthetic pyrethroid type II. Deltamethrin result cannot be compared as toxicant and fish are different. But the report emphasized the utility of micronuclei assay to detect the genotoxicity.

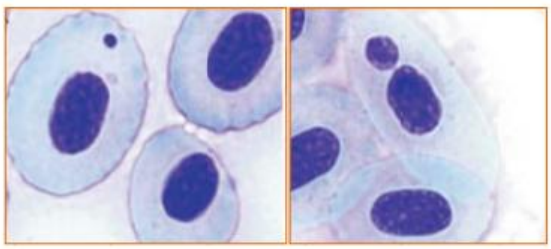
Renu Chaudari and Saxena (2016) reported geno-toxicological assessment of pyrethroid Bioallethrin in freshwater fish *Channa punela*. Biallethren, type I pyrethroid toxic to fish and is more at lower temperature and is more toxic to cold than warm waters. The micronucleus test is performed in three sublethal concentrations and DNA damage is noticed, which can lead to clastogenecity of toxicant. The micronuclei increased upto 10 days and after that the frequency decreased. They concluded that MNT test is useful for detecting the changes in the fish and this is maker for genotoxicity. The result can be compared with the present study as the methodology of exposure is different and only serve as indices of genotoxicity – which will serve fish using as model species for environmental biomonitoring studies.

Table 2: MNT observations in the individual blood cells

Control	Lethal				Sublethal			
	TG	%	11% EC	%	TG	%	11% EC	%
	0	0	0	0	0	0	0	0
	1	01	2	0.2	0	0	0	0

Percentage of micronuclei cells in blood of *Ctenopharyngodon idella* exposed to lethal and sublethal concentrations of Technical grade and 11% EC of Deltamethrin.

Fig. 1 A&B. Micronucleus observed after the exposure of Fish – *Ctenopharyngodon idella* for Technical Grade and 11% EC of Deltamethrin at lethal concentration.



Fulya and Güner (2011) reported induction of micronuclei following exposure to pyrethroid insecticide lambda cyhalothrin, another type of type I synthetic pyrethroid to the mosquito fish *Gambusia affinis*. The fish were exposed to for a periods of 6, 12, 24 and 48 h at two different sublethal concentrations ($1x10^{-4}$µg and $2x10^{-4}$µg). The toxicant induced Micronucleus in erythrocytes in the early duration and decreased after 24-48 treatment of $4x10^{-4}$µg. They concluded that the toxicant has genotoxic potential. The result can be not correlated with the present study because of difference in methodology of exposure is different and can presumed that it will be useful biomarker.

Another group I synthetic pyrethroid Bifenthrin in the zehra fish *Danio rerio* gill tissue assessed by the exposure and damage to DNA is observed (Rajini *et al*., 2015).

Omer Saylar (2016) reported toxic effects of permethrin on Pseudoresbora parva. The fish after ecxposure to 1/10 of LC_{50} value for four days and reported Micronucleus differences.

The study concluded that genotoxicolocial biomarker of toxicity in fish is a useful indicator of environmental pollution. The study when compared with, whether belong to type I or type infact type II behaved in the same manner and serve as a tool for evaluation of genotoxicity.

Ansari *et al*., (2011) reported *in vivo* cytogenetic and oxidative stress inducing effects of Cypermethrin in fresh water fish *Channa punctatus* (Bloch). The fish exposed to Cypermethrin 0.4, 0.8 and 1.2 µg/l for 48 and 72 hours showed increased frequency of chromosomal aberration and micronucleus in a concentration dependent manner which is due to increased oxidative stress and disturbances of antioxidant enzymes.

Yester Kahn et al 2012 reportedthe vitamin E role which had a role in the reduced frequency of micronuclueus when deltamethrin is used as toxic ant only. This concept of idea is good and can be practiced in culture practices as diet supplement. The adult fish were exposed to three concentrations of technical grade deltamethrin 0.4, 0.8 and 1.2 micro g/L for 48 h and 72 h. Deltamethrin significantly induced micronucleus via the oxidative stress and it serves as a good biomarker. The present study with technical grade only MN was observed which serves as a biomarker of toxicity in evaluation, which was not observed in sublethal and also in EC 11% Deltamethrin.

Ibrahim EI – Elaimy *et al*., (2014) reported oxidative stress that lead to inbance of osmotic, activity which are all due to paved by the way genotoxic action.

Jaya Sahi and Ajay Singh (2014) reported genotoxic and haemotological effect of commonly used fungicide manolozeb in terms of micronucleus assay on the fish *Clarias batracus*. Fish were exposed to sublethal concentration 80% of LC_{50} of 24h of Mancozes and the members of micronuclei at 48 h were maximum.

Bucker *et al*., (2012) reported Micronucleus test of erythrocytes of the Amazonian electric fish *Apternotus bonaparti* exposed to Benzene. Blood samples were collected at 0, 24, 48, 72 and 96 hours of exposure at 10 and 25 ppm concentration of the toxicant. At lower concentrations MN was higher after exposure to 48 h of the toxicant and do not show change in zero to 96 h. The study concluded that the investigation is a biological model for biomonitoring purposes in the Amazon and as a suitable gentoxic marker.

Nwani *et al*., (2011) reported on MN in *Channa punctata* using or toxicant Atrazine herbicide exposed to three sublethal concentrations. Micronuclei induction in erythrocytes was highest on day 7 of exposure. The study concluded that MNT is a useful test in determining potential genotoxicity of which pollutants, which might be appropriate as a part of monitoring programme.

Vasanth and Subrahmanyam (2016) reported genotoxic effect of Atrazine on *Poecilla sphenops* using micronuclear assay. The fish were exposed to three concentrations 2.5, 1.25 and 0.83 µg/L for 30 days. A significant increased in the frequencies of micronuclei in erythrocytes of the fish tested and explained the genotoxic potential of this toxicant.

Faiza and Zaveen (2018) reported pesticide induced DNA damage in the peripheral blood erythrocytes of freshwater fish *Oreochromis niloticus*. The pesticide mixture endosulphan and chloropyriphos exposed to the fish in the erythrocytes of the blood lead to DNA damage. The study concluded that careful and sensible use of pesticides to guard against genetic hazards.

Ahrar Khan *et al*., (2012) in their review article haemato-biochemical changes induced by pyrethroid insecticides in Avian, Fish and mammalian species reported that, the toxicants as pyrethroids induced DNA damage which appear in the form of micro-nucleus formation. They also referred that micronucleus appearance in the cytoplasm is considered as biomarker of genotoxicity.

Sunanda *et al*., (2016); Sana Ullah and Jallil (2015), Hasibur Rehaman (2014) and Sankar murthy *et al*., (2013) mentioned in their review articles that pesticides organochlorines, organophosphates and synthetic pyrethroid

induce genotoxicity and serve as biomarker of fish toxicity in the studies of Ecotoxicology.

Anilava Kaviraj and Abik Gupta (2014) reported genotoxic effets as biomarkers where in MNT could gainfully used as a specific biomarker due to pesticide toxicity.

Not only pesticides, even metals like methyl mercury in fish, by MNS s considered as a tool for genotoxicity (Carlos Alberto 2009).

Kaushik *et al*., (2015) in their review article mentioned about the genotoxic studies in fish.

Thus as above discussed, the fish ecotoxicology have many biomarkers of type II synthetic pyrethroid pesticides in fresh water fish. Haematological biomarkers hyperglycemia as a biomarker, Enzymes of energy metabolism as biomarkers, oxidative stress biomarkers. Enzymes of nitrogen metabolism as biomarkers, AChE activity as biomarker, gene expression as biomarker genotoxic effects as biomarkers, omic biomarkers and specificity in sodium channel interactions – are the several biomarkers mentioned.

However in the study of preliminary type of MNT test is taken with certain limitations of further advancement of the research in the lines. But the fish *Ctenopharyngodon idella* in the present study wherein MNT was done may be because of the google is silent over the such types of studies using synthetic pyrethroid.

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