

NUCLEAR ABNORMALITY AND MICRONUCLEI INDUCED UNDER THE EFFECT OF INSECTICIDE TARA AFTER THREE DAYS OF TREATMENT

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Summary

Kurteshi, K., Z. Shaqiri, K. Letaj & M. Hoxha, 2013. Nuclear abnormality and micronuclei induced under the effect of insecticide tara after three days of treatment. *Bulg. J. Vet. Med.*, 16, Suppl. 1, 46–49.

The aim of this study is to determine the frequency of micronuclei and nuclear abnormalities in peripheral erythrocytes of fish *Carassius aureatus*, after treatment with insecticide tara (generic name thiamethoxam – 3-[(2-Chloro-1,3-thiazol-5-yl)methyl]-5-methyl-*N*-nitro-1,3,5-oxadiazinan-4-imine). Fish were treated with four different concentrations of the insecticide for 3 days. The frequency of micronuclei and nuclear abnormalities found, was significantly higher in treated group, compared with control group.

Key words: erythrocyte, genotoxic effect, fish, insecticide, micronuclei, tara

INTRODUCTION

The presence of micronuclei and nuclear abnormalities in peripheral blood erythrocytes has been used as a biomarker of genotoxicity caused by pollutants since 1970's (Schmidt, 1975). One of the advantages for their use is that 97% of fish blood cells are red blood cells, and only 3% are white blood cells, so this represents a high homogeneity (Mitchellmore & Chipman, 1998).

The presence of micronuclei in peripheral blood fragments or chromosomes delays, in relation to others their migration to the poles of the cell in anaphase (Heddle, 1973; Schmidt, 1975; Al-Sabti & Metcalfe, 1995). According to Heddle *et al.* (1991) and Al-Sabti & Metcalfe (1995), micronuclei can also be formed by apoptosis, inactivation of the spindle formation and chromosome damage beyond

action of physical agents. Other abnormalities in the nuclei of red blood cells have been mentioned as biomarkers of cyto- and genotoxicity and thus used in a complementary analysis of the frequency of micronuclei.

MATERIAL AND METHODS

We used one species of fish, *Carassius auratus*. Golden fish (*Carassius auratus*) was chosen for this study because they were very adaptive for investigation, also due to their proven sensitivity to genotoxic chemicals. The fish were collected in the lake Vasileva, central part of Kosovo. After the capture, they were placed in aquaria with aerated tap water and taken to the laboratory. After acclimatisation to reduce the stress of capture and transport,

fish were treated in aquaria with insecticide for 3 days or 72 hours.

Slides were stained with May-Grunwald-Giemsa. The frequency of erythroblasts, micronuclei and nuclear abnormalities were estimated by counting 2,000 cells in extensions. Ten fish were put in each aquarium; the total number of fish was 50.

Experimental design

Carassius auratus fish were placed in five different aquaria, one containing tap water (negative control) and four aquaria containing different dilutions of insecticide tara. The concentration of insecticide tara (generic name thiamethoxam – 3-[(2-Chloro-1,3-thiazol-5-yl)methyl]-5-methyl-N-nitro-1,3,5-oxadiazinan-4-imine) in the first aquarium was 15 mL insecticide/40 L water, in the second aquarium – 12 mL insecticide/40 L water, in the third aquarium – 10 mL insecticide/40 L water, and in the fourth aquarium – 8 mL insecticide/40 L water. The fifth aquarium was used as control, without insecticide tara, and contained only drinking water.

Slide preparation and staining

The fish was cut in caudal region and smears of peripheral blood were made on free clean slides. For each fish three slides

were prepared. Slides were coded for each fish. The smears were air-dried and fixed in absolute ethanol for 25 min. After fixation, the slides were stained in aqueous Giemsa (diluted in destiled water ratio 1:3) for 45 min.

RESULTS

The frequency of nuclear abnormality (NA) and micronuclei (MN) induction is shown in Tables 1 and 2. Significantly more induced MN and NA in peripheral blood erythrocytes were detected in all treatment groups, compared with control untreated group.

As shown in Table 1, the NA number was higher in the first aquarium – 13, while at second aquarium was 8. In the third and fourth aquaria the same number of nuclear abnormalities was found (7), about twofold more than in the control group of fish. Average NA number for all treated aquaria was 8.75 NA per 1,000 erythrocytes. NA in all groups treated with the insecticide statistically were significantly higher compared with the control group ($P < 0.001$).

The frequencies of micronucleated erythrocytes were estimated for each fish in each aquarium. In the first aquarium we

Table 1. Average number (per aquarium) of nuclear abnormalities (NA) in 2,000 peripheral blood erythrocytes of *Carassius auratus* fish after 3-day treatment with different concentrations of the insecticide tara

Aquarium	Average number of NA/2,000 erythrocytes per aquarium	Significance
Aquarium 1 (15 mL fungicide /40 L water)	13	$P < 0.001$
Aquarium 2 (12 mL fungicide /40 L water)	8	$P < 0.001$
Aquarium 3 (10 mL fungicide /40 L water)	7	$P < 0.001$
Aquarium 4 (8 mL fungicide /40 L water)	7	$P < 0.001$
Aquarium 5 (control)	3	
Average number of NA in treated fish, without the control group	8.75	

Table 2. Average number (per aquarium) of micronuclei (MN) in 2,000 peripheral blood erythrocytes of *Carassius auratus* fish after 3-day treatment with different concentrations of the insecticide tara

Aquarium	Average number of MN/2,000 erythrocytes per aquarium	Significance
Aquarium 1 (15 mL fungicide /40 L water)	51	P<0.001
Aquarium 2 (12 mL fungicide /40 L water)	43	P<0.001
Aquarium 3 (10 mL fungicide /40 L water)	36	P<0.001
Aquarium 4 (8 mL fungicide /40 L water)	22	P<0.001
Aquarium 5 (control)	5	
Average number of NA in treated fish, without the control group	38	

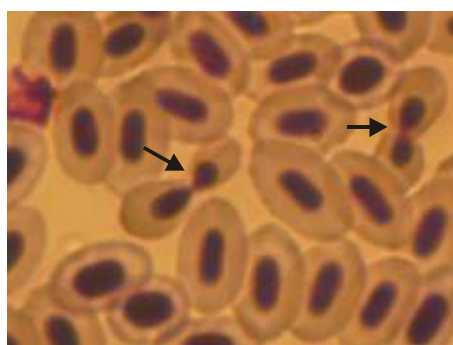


Fig. 1. Nuclear abnormalities (arrows).

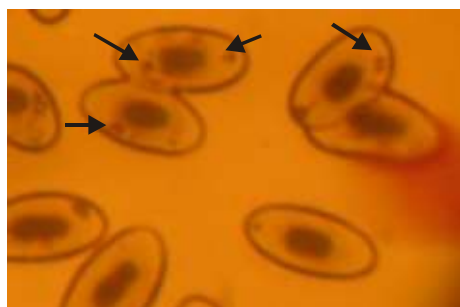


Fig. 2. Micronuclei (arrows).

detected 51 micronuclei which was higher compared with other aquaria and with control group.

In the second aquarium we determined 43 MN, while the third and fourth aquaria has 36, respectively 22 MN/1000 erythro-

cytes. The average number of MN at all groups treated with insecticide was significantly higher compared with control group(P<0.001)

DISCUSSION

Fish are in an intimate contact with their environment which contains high concentrations of different biological, chemical, physical pollutants.

When a toxic substance is introduced into the aquatic environment, three main steps can be identified before a response is produced from an aquatic organism (Tao *et al.*, 1999): 1) chemical and physico-chemical processes, in which the substance interacts with other constituents of the water and becomes available to the organism; 2) physiological processes, including absorption, transport, distribution, metabolic transformation, accumulation, and 3) excretion and intoxication processes, including combination with receptors. In the present study the experimental conditions were stable to avoid differences of metal accumulations in fish.

Our findings are in concordance with the results of other authors, such as Matsuoto & Colus (2000); Grisolia & Cordeiro (2000); Ayllon & Garcia-Vazquez

(2001); Rodriguez- Cea *et al.* (2003) who notified the induction of micronuclei by several well-known clastogenic/mutagenic agents such as cyclophosphamide, mitomycin-C, bleomycin, colchicine, ethyl methanesulphonate and vinblastin, assessed in freshwater and marine fish species.

The results of our study are also in accordance with results of Buschini (2004), who exposed *Cyprinus carpio in situ* to lake waters treated with disinfectants for potabilization.

CONCLUSIONS

Based on this investigation we can conclude that the insecticide tara damaged the chromosomes of erythrocytes of *Carasius auratus*. The found frequency of micronuclei and nuclear abnormalities was significantly higher in treated groups, compared with controls.

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