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Micronucleus test of Californian trout fish after treatment with the herbicide monosan for 48 and 96 hours

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Abstract:

Aim: Fish are the object of study to detect potential contaminants, Mutagenic or carcinogenic present in the water. Besides fish toxicant similar response can provide higher vertebrates for chemicals that are carcinogenic to humans. Micronucleus test (MNT) is a technique developed by Schmid (1975), using the cells as an object of study, and by treating with chemicals as a genotoxic test. Micronucleus test can be used to fish, frogs and birds, and is a biological warning direct or indirect contamination of aquatic environment in vivo. **Research goal**: The purpose of this research was verified genotoxic effect of herbicide monosan in peripheral blood erythrocytes of trout fish of California (Oncorhynchus mykiss), thus to prove damage to the genetic material (chromosomes) in erythrocytes by MN test. **Results**: It's seen that the frequency of micronucleus (MN) in erythrocytes of fish treated for 96 hours in all concentrations (0.2, 0.3, 0.4 and 0.5 ml / L), is in scale significantly higher ($p < 0.001$) compared with the control group of fish. The high frequency with micronucleus in significant degree is also determined to fish treated for 48 hours in all dilutions (0.2, 0.3, 0.4 and 0.5 ml / L) compared with the control group. **Conclusion**: In our research we found a significant increase ($p < 0.001$) the frequency of MN in peripheral blood erythrocytes of fish treated with herbicide monosan, compared with control group of fish.

Key words: Micronucleus test, herbicide monosan, Californian trout fish.

Introduction

A micronucleus test is a test used in toxicological screening for potential genotoxic compounds. The assay is now recognized as one of the most successful and reliable

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assays for genotoxic carcinogens, i.e., carcinogens that act by causing genetic damage and is the OECD guideline for the testing of chemicals.

Fish are the object of study, to detect potential contaminants, mutagenic or carcinogenic present in the water. Besides fish, toxicant similar response can provide higher vertebrates, for chemicals that are carcinogenic to humans. Micronucleus test (MNT) - is a technique developed by Schmid (1975), using the cells as an object of study, as well as treated with chemicals such as genotoxic test. Micronucleus test can be used to fish, frogs and birds, and is a biological warning direct or indirect contamination of aquatic environment in vivo. Micronucleus produced by fragments of chromosomes or whole chromosomes, which remained behind during cell division, the remaining back (delayed) because you lack centromeres, or have damaged or have defects centromeres in cytokines. During cell division, the shape, size and number of chromosomes are the same, and divided equally between the daughter cells. If during cell division, chromosomes are damaged or cut off by the action of various substances toxic, then the distribution of genetic material between two daughter cells is not equal, because the chromosomes of all or part of them may remain outside nucleuses cells daughters then the material is rolled membrane forming a micronucleus, which can be clearly seen under the microscope. MN diameter can be as 1/3 of the main nucleus has oval or round, MN should be separate from the main nucleus, so as to clearly distinguish their borders, MN should have similar color to the main nucleus. MN in vivo and in vitro, shows damage to the chromosomes or the mitotic apparatus in the body's cells as a result of the action of various factors, chemical, physical and biological environment in which living organisms. The main abnormality that can be determined with the micronucleus test is: rupture of chromosomes, this difference may be due to chronic damage to chromosomes.

Research goal

The purpose of this research was verified genotoxic effect of herbicide monosan in peripheral blood erythrocytes of trout fish of California (Oncorhynchus mykiss), thus to prove damage to the genetic material (chromosomes) in erythrocytes by MN test.

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Material and methods

Monosan herbicide testing is done in laboratory conditions. Ten fish were placed in eight aquariums (a total of 80 fish treated), in which are previously thrown out 50 L of water, and the concentration of certain herbicide monosan (0.2, 0.3, 0.4 and 0.5 ml / L). Four aquariums were treated by 48 hours, and four other aquariums were treated by 96 hours. Control group of fish (10 fish) are placed in an aquarium which contained no herbicide.

Before it was taken blood from the caudal vein, it is measured weight and height, expressed in grams (weight), and centimeters (length). Blood was taken from the caudal vein of each fish. Decided by a drop of blood on the glass object, glass and other assistance be extended blood (for each fish are done by four stretch-painting). From each fish preparations were prepared by 4, then total 360 preparations. The painting was made as thin in order to be scattered red blood cells, and to enable a much better view. Drying is making preparations for two hours. Fixation of products is made with 96% absolute ethanol for 20 min. Painting (the method of Schmid's 1975) preparations is made with solvents to May-Grenwald's diluted with distilled water in the ratio 1: 1 for 25 minutes, then painting with tincture of Giemsa diluted with distilled water in ratio of 1: 6 (Giemsa stains nuclear material colored dark significantly compared with cytoplasmic material), for 25 minutes. Rinse the preparations was made with distilled water, which are left to dry (dry) for 30 minutes. Erythrocyte survey is done under a microscope, with 400 times magnification, in each preparation is decided by a point cedar oil (see more clearly that the cells). Count of micronucleus in erythrocytes of peripheral blood is made, counting how micronucleus were in 2000 erythrocytes for fish, as for every fish are prepared by four preparations of each preparation are numbered from 500 erythrocytes, and the number of possible presentation of micronucleus.

Statistical methods

Statistical processing of the results is made with SIGMA STAT 3.0 statistical software, version 2004.

We found the arithmetic average weight (X), length (X), micronucleus (X), standard deviation (SD), significant (p) and t-test. Statistically they analyzed the following parameters: weight, height, and number of micronucleus.

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Results

Table 1. The frequency of MN in peripheral blood erythrocytes analyzed in 2000 erythrocytes for fish, the fish of California trout (Oncorhynchus mykiss), after treatment for 48 and 96 hours at various dilutions.

Fish treatments	Monosan herbicide dilution with water (ratio)	The average body length (cm) 10 fish	The average body weight (g) 10 fish	Average MN for a treatment (X)	Standard deviation	Significance (between groups of fish treated and control group)
Treatment I after 48 hours	0.5ml:1L	18.9 cm	68 g	2.3 MN	±1.49	P=<0.001
Treatment II after 96 hours	0.5ml:1L	19 cm	73 g	3.1 MN	±1.45	P=<0.001
Treatment I after 48 hours	0.4ml:1L	17.4 cm	67 g	1.9 MN	±1.73	P=0.007
Treatment II after 96 hours	0.4ml:1L	18.6 cm	81 g	2.6 MN	±1.50	P=<0.001
Treatment I after 48 hours	0.3ml:1L	19.35 cm	114 g	1.4 MN	±0.84	P=<0.001
Treatment II after 96 hours	0.3ml:1L	17.5 cm	55 g	2.2 MN	±1.03	P=<0.001
Treatment I after 48 hours	0.2ml:1L	18.45 cm	80.5 g	0.9 MN	±0.56	P=0.006
Treatment II after 96 hours	0.2ml:1L	18.5 cm	84.5 g	1.5 MN	±0.70	P=<0.001
Control fish	----------	17.9 cm	77.5 g	0.2 MN	±0.42	----------

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Table 2. Statistical analysis (T-test and Significance) the average frequency of MN, the fish treated at various dilutions, and while various treatments, which are a significant degree.

Comparisons between	The average frequency for treated MN	T-test	Significance	S -is significant
0.5ml/96h: Control	3.1 : 0.2	t = -6.076	P= <0.001	S
0.5ml/48h: Control	2.3 : 0.2	t = -4.277	P= < 0.001	S
0.4ml/96h: Control	2.6 : 0.2	t = -4.854	P= < 0.001	S
0.4ml/48h: Control	1.9 : 0.2	t = -3.021	P= 0.007	S
0.3ml/96h: Control	2.2 : 0.2	t = -5.669	P= < 0.001	S
0.3ml/48h: Control	1.4 : 0.2	t = -4.025	P= < 0.001	S
0.2ml/96h: Control	1.5 : 0.2	t = -4.993	P= < 0.001	S
0.2ml/48h: Control	0.9 : 0.2	t = -3.130	P=0.006	S
0.3ml/96h:0.2ml/48h	2.2 : 0.9	t = 3.488	P=0.003	S
0.4ml/96h:0.3ml/48h	2.6 : 1.4	t = 2.199	P=0.041	S
0.4ml/96h:0.2ml/48h	2.6 : 0.9	t = 3.341	P=0.004	S
0.5ml/48h:0.2ml/48h	2.3 : 0.9	t = 2.769	P=0.013	S
0.5ml/96h:0.3ml/48h	3.1 : 1.4	t = 3.206	P=0.005	S
0.5ml/96h:0.2ml/48h	3.1 : 0.9	t = 4.470	P= < 0.001	S
0.5ml/96h:0.2ml/96h	3.1 : 1.5	t = 3.138	P=0.006	S

As shown in Table 2. micronucleus frequency differences in scale significantly ($p < 0.001$), have shown all groups of fish treated at different concentrations of herbicide treatments in both time (48 and 96 hours) compared with the control group.

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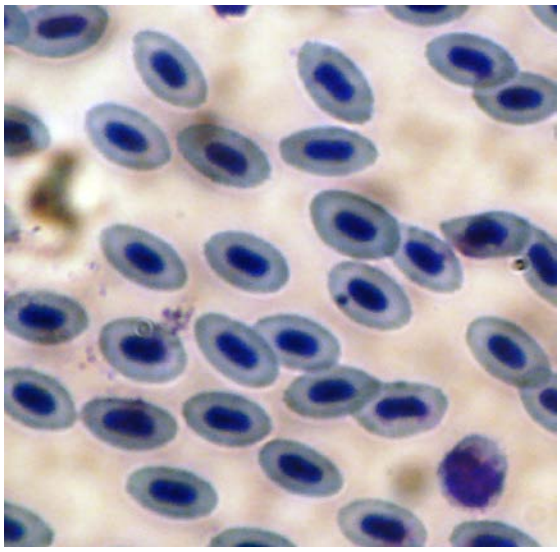
The high frequency of micronucleus significant scale is also found in fish treated concentration of 0.4 ml / L for 96 hours, compared to fish treated in concentration 0.2 and 0.3 ml / L for 48 hours.

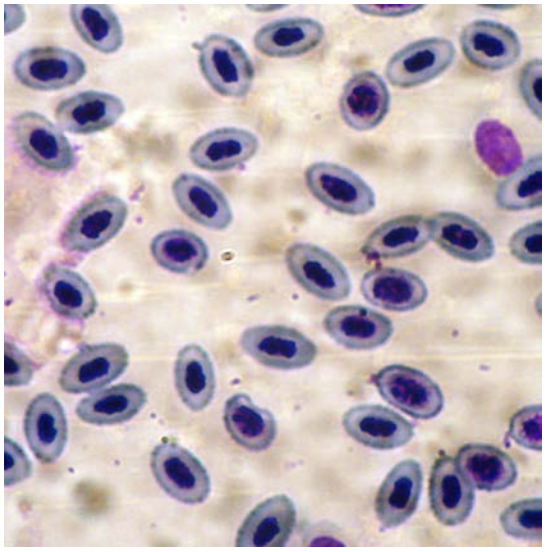
Micronucleus frequency is higher in the fish ladder is also significant in the concentration of treating 0.3 ml / L for 96 hours, compared to fish treated in concentration 0.2 ml / L for 48 hours.

Micronucleus frequency is higher in the fish ladder is also significant in the concentration of treating 0.5 ml / L for 96 hours, compared to fish treated in concentration 0.2 and 0.3 ml / L for 48 hours.

Also higher frequency of micronucleus in significant scale is also observed in fish treated herbicide concentration of 0.5 ml / L for 48 hours, compared to fish treated in concentration 0.2 ml / L for 48 hours.

Micronucleus high frequency of significant scale has also been observed in fish treated at a concentration of 0.5 ml / L for 96 hours, compared to fish treated in concentration 0.2 ml / L for 96 hours.





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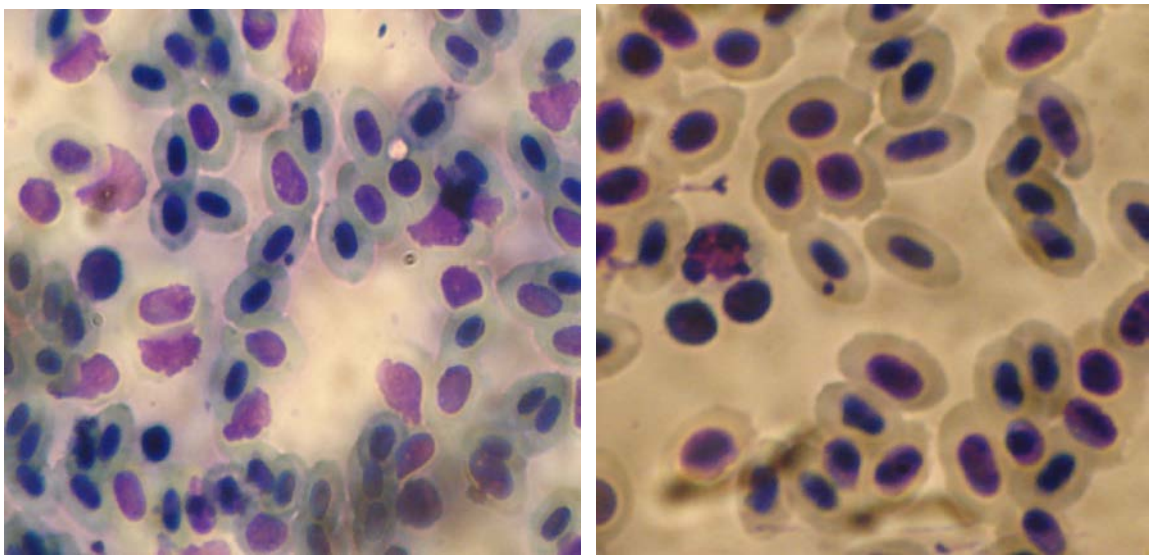

Discussion

In our research we found a significant increase ($p < 0.001$) the frequency of MN in peripheral blood erythrocytes of fish treated with herbicide monosan, compared with control group of fish.

Our results are consistent with the results obtained from Barshiene and co., (2007), which detected a significant increase in the frequency scale MN in erythrocytes, to fish trout California (Oncorchynchus mykiss) after treatment for 14 days, with the blend of heavy metals.

Our results are consistent with results obtained by Ateeq Bushra and co., (2002), who detected increased in scale significantly the frequency of MN in erythrocytes, the fish (Clarias batrachus) after treatment for 48, 72 and 96 hours, two herbicides: 2,4 dichlorophenoxyacetic acid and butachlor.

Our results are consistent with results obtained by Serpil Cones and Tolga Qavash (2008), who detected increased in scale significantly the frequency of MN in erythrocytes, the fish (Oreochromis niloticus), after treatment for 3, 6 and 9 days herbicides and Treflan Trifluralin.

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High frequency of micronucleus significant scale in peripheral blood erythrocytes of fish (Prochilodus lineatus), after treatment for 6 and 96 hours, with the herbicide Roundup, have found DGSM Calvante and co., (2008).

Similar results obtained Jorge, IR and co., (2004), who detected increased in scale significantly the frequency of MN in erythrocytes, the three types of fish Amazonian (Prochilodus nigrikans, Mylossoma duriventris and Hoplias malabaricus), after treatment with mercury .

Marcela Alejandra Campana and co., (2003), have detected a significant increase in scale of the MN frequent in erythrocytes, the frog (Rana catesbeiana) after treatment for 24, 48 and 72 hours with pyrethroid insecticide.

Also Tolga Qavash and Serpil Conen (2007), have detected a significant increase in scale of the MN frequent in erythrocytes, the fish (Carassius auratus), after treatment for 48, 72 and 96 hours with the herbicide glyphosate.

High frequency of micronucleus significant scale in peripheral blood erythrocytes of fish (Heteropneustes fossilis), after treatment with pentachlorophenol for 48, 72 and 96 hours, has won Ahmad and co,. (2002).

Similar results have also won Barshiene J and co., (1996), who detected increased in scale significantly frequency micronucleus in erythrocytes trout Brown (Salmo trutta fario) after treatment for 3, 6, and 14 days with biphenyl polychlorinated .

CONCLUSIONS

According to research conducted on the genotoxic effect on the frequency of herbicide monosan micronucleus in peripheral blood erythrocytes of trout fish of California (Oncorchynchus mykiss), the following conclusions:

MN high frequency of significant scale, the fish treated for 48 and 96 hours, in all dilutions (0.2, 0.3, 0.4 and 0.5 ml / L) compared with the control group.

MN high frequency of significant scale, the fish treated for 96 hours, in dilution of 0.4 ml / L, compared with fish treated for 48 hours at dilutions 0.2 and 0.3 ml / L.

MN high frequency of significant scale, the fish treated for 96 hours, in dilution 0.3 ml / L, compared with fish treated for 48 hours at dilutions 0.2 ml / L.

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MN high frequency of significant scale, the fish treated for 96 hours, in dilution of 0.5 ml / L, compared with fish treated for 48 hours at dilutions 0.2 and 0.3 ml / L.

High frequency of MN-scale significant, fish treated for 48 and 96 hours, in dilution of 0.5 ml / L, compared with fish treated for 48 hours, in dilution 0.2 ml / L and 96 hours a slimming 0.2 ml / L .

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