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Full Length Research Paper

The intensity of pollutant genotoxicity in Lake Uluabat: Investigation of the peripheral erythrocytes of *Cyprinus carpio*

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Clastogenic effects of home wastes and agricultural contaminates of Lake Uluabat ("40°11'41.38"N, "28°27'08.40"E) were investigated in peripheral erythrocytes of *Cyprinus carpio*. Examination of blood smears showed that the formation of micronuclei was significantly increased (f = 21.89, df = 3) with p value > 0.001during one year, and where more abundant in the same species of fish compared to the control. This increase in the formation of micronucleus indicates that home waste and agricultural pollution increase the clastogenic effects on peripheral erythrocytes of *C. carpio* and may have similar effects on the human population located around the lake and consume the fishes.

Key words: Clastogenic effects, *Cyprinus carpio*, environmental genotoxicity micronucleus test.

INTRODUCTION

The micronucleus (MN) test is an *in vivo* and *in vitro* short-time screening test, developed by Schmid (1975) and Handle (1973) which is widely used to detect genotoxic effects (Villarini et al., 1998). It is one of the simple, reliable, least expensive and rapid screening system for both clastogenic (chromosome breakage, formation of a centric fragments) and eugenic (chromosome lagging and effects on spindle) effects (Heddele et al., 1983; Orhan et al.; 1993). Clastogenic and eugenic agents affect the spindle apparatus, which can be differentiated on the basis of the relative induced MN sizes or with the presence of kinetochores (Heddle et al., 1983; Yamamoto and Kikuchi, 1980; Heddle et al., 1991).

In anaphase, any chromosome fragment or whole chromosomes which lack a centromere may not be integrated in the nucleus due to lack of an indispensable element for orientation in the spindle apparatus. After telophase, the fragments or whole chromosomes give rise to one or several secondary nuclei which are smaller than main daughter nucleus and are therefore called micronuclei (Schmid, 1975; Heddle et al., 1973).

The usefulness of the MN test for mutagenicity screening has been well established in several systems, which include the ovary, bone marrow, epithelial tissue, peripheral blood, liver, exfoliated buccal cells and fetus cells of several laboratory animals or human (Heddle, 1993; Krishna et al., 1991; Konopacka et al., 1998; Agrawal, 1999; Saleh and Zeytinoglu, 2001). Micronuclei formation can occur in any dividing tissue of any species (Haddle et al., 1983) as shown by the values of spontaneous micronucleated erythrocytes (MNE) or some laboratory animals and mammals (Zuniga et al., 1996). Some organisms such as plants, fish, birds and frogs were also investigated by MN test to detect environmental pollution capable of producing genotoxic damage (Ma et al., 1984; Bhunya and Jena, 1993; Zhuleva et al., 1996; Chu et al., 1996).

Hayashi et al. (1998) evaluated monitoring systems that use aquatic organisms to assess the genotoxicity of water in the field and in the laboratory. In a field study, MN assay was shown to be applicable to freshwater and marine fishes and that gill cells are more sensitive than hematopoietic cells to micronucleus inducing agents. Aquatic animals have often been used in bioassays to monitor water quality of effluent and surface water (Siboulet et al., 1984; Fernandez et al., 1993; Saleh and Zeytinoglu, 2001). The development of biological monitoring techniques based on fish offers the possibility of checking water pollution with fast responses on low concentrations of direct acting toxicants (Andrade et al., 2004; Al-Sabti

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and Metcalfe, 1995).

In the present study, we have investigated the clastogenic effects of agricultural and human waste contaminants in the lake Uluabat on the formation of MN in erythrocytes of peripheral blood of lake fish *Cyprinus carpio*.

MATERIALS AND METHODS

Animals

Healthy and active *C. carpio* were collected from the Uluabat Lake near Bursa City (Turkey) over a year and four months, thrice every year (five fishes for each collection). The weight of fish ranged from 200 - 280 g, with mean length of 17 ± 3 cm. The control group includes small larvae of the same fish species (15 individuals) collected from the same lake and grown in the laboratory aquarium (150 × 50 × 30 cm^3) until they reach the target weight, maintained under standard conditions temperature (27 ± 2) and were supplied with standard food purchased freshly from shop markets and filtered fresh water. Water was changed every 3 days, while the other three groups were collected freshly from the lake located at Uluabat every 4 months. Venous blood was collected from gills of each fish in a heparinized tubes.

Slide preparation and staining

For each fish, three microscopic slides were prepared. Clean slides were taken and fresh blood samples from each fish collected from the gills in heparinized tubes were smeared onto the slides for experimental and control groups. The slides were air dried for 30 min and then fixed in cold Corney fixative for 10 min. After fixing, the slides were stained in aqueous Giemsa (5%) (Sigma) for 10 min and May-grunwald Giemsa (Sigma) for 2 min (Saleh and Zeytinoglu, 2001).

Examination of slides

Five fishes were used for each sample and control; 6,000 cells/Fish were analyzed, totaling 30,000 erythrocytes/sample. The frequencies of micronuclei in erythrocytes were detected under a binocular microscope (OLYMPUS) using a 1000× oil-immersion lens. Only cells with intact cellular and nuclear membranes were scored. The following criteria were used as described by previous studies: (i) micronuclei should be one-tenth and one-third diameter of the main nucleus, (ii) they should be on the same plane of focus, (iii) they should have the same color, texture and refraction as the main nucleus, (iv) they should be clearly separated from the main nucleus. Micronuclei formation showed variation in shapes and number per cell.

Statistical analysis

The present data were statistically analyzed by one-way ANOVA. The variance was considered significant at $p < 0.05$.

RESULTS

This study of the clastogenic effect of Lake Uluabat fresh water using MN revealed that there was a significant induction of MN in *C. carpio*. The results showed that the control group had the lowest value of MNE, while the MNE increased significantly with the time in the other three groups (Tables 1 - 4). All groups were significantly increased when compared to control ($f = 21.89$, $df = 3$), whereas, this increase was not very significant between group one and group two ($f = 0.679$, $df = 1$). Slight increase was also detected between group two and group three ($f = 1.52$, $df = 1$). Whereas, it was more significant when group one was compared with group three ($f = 10.03$, $df = 1$) (Table 5).

The microscopic investigation of micronuclei also showed a variation in their shapes and number per cell as shown in Tables 3 and 4 and Figure 1. The MN type (A) was found in all groups, while, type (B) and (D) MN were found abundantly in groups 2 and 3 (Figure 2).

DISCUSSION

Bioindicators offer several types of unique information that are not available from other methods: (1) Early warning of environmental damage; (2) the integrated effect of a variety of environmental stresses on the health of an organism and the population, community, and ecosystem; (3) relationships between the individual responses of exposed organisms to pollution and the effects at the population level; (4) early warning of potential harm to human health based on the responses of wildlife to pollution; and (5) the effectiveness of remediation efforts in decontaminating waterways. Villela et al. (2006) and Al-Sabti and Metcalfe, (1995) showed that 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 waterborne pollutants. The sampling of peripheral blood is appropriate and sufficient for biomonitoring projects. Lyne et al. (1992) and Cesar and Célia (2000) demonstrate that *Tilapia rendalli* and *Oreochromis niloticus*, give different responses to genotoxic agents. According to clastogen and the species studied, the frequency of micronuclei may suffer important variations. Time-dependent responses have also been observed in amphibians exposed to radiation. Siboulet et al. (1984), Fernandez et al. (1993) and Ali et al. (2008) confirmed that the micronuclei frequencies may vary according to the season, the kind of pollution involved and the species of fish. It was concluded from these studies that fish can be used for estimating the genotoxic effects of water-borne pollutants.

Different references showed that MNE can be affected by many factors such as age, sickness, species, feeding, chemical and physical agents and environmental conditions (al-Sabti and Metcalfe, 1995; Saleh and Zeytinoglu, 2001). So to eliminate these factors, healthy, young and active individual had been chosen from the same species, *C. carpio*.

During the present study, it has been demonstrated that

Table 1. Number of micronucleated erythrocytes (MNE) observed in *C. carpio* (control group).

Animal			Slide No.	*Ery. No*	Micronucleus type			
No.	**L. cm**	**Wg. gr**			**A**	**B**	**C**	**Total**
1	17	210	1	2000	2	0	0	
			2	2000	2	0	0	
			3	2000	1	0	0	
Total			**3**	**6000**	**7**	**0**	**0**	**7**
2	21	224	1	0	0	0	0	
			2	0	2	0	0	
			3	0	3	0	0	
Total			**3**	**6000**	**5**	**0**	**0**	**5**
3	23	230	0	0	2	0	0	
			0	0	3	0	0	
			0	0	1	0	0	
Total			**3**	**6000**	**6**	**0**	**0**	**6**
4	28	256	0	0	3	0	0	
			0	0	2	0	0	
			0	0	2	0	0	
Total			**3**	**6000**	**7**	**0**		**7**
5	15	204	1	0	1	0	0	
			2	0	2	0	0	
			3	0	0	0	0	
Total			**3**	**6000**	**3**			**3**

Table 2. Number of micronucleated erythrocytes (MNE) observed in *C. carpio* (Period 1).

Animal			Slide No.	Ery. No	Micronucleus type			
No.	**L. cm**	**Wg. gr**			**A**	**B**	**C**	**Total**
1	22	230	1	2000	4	0	0	
			2	2000	5	0	0	
			3	2000	3	0	0	
Total			**3**	**6000**	**12**			**12**
2	29	253	1	2000	6	0	0	
			2	2000	5	0	0	
			3	2000	0	0	0	
Total			**3**	**6000**	**13**			**11**
3	33	268	1	2000	1	0	0	
			2	2000	5	0	0	
			3	2000	8	0	0	
Total			**3**	**6000**	**14**			**14**
4	35	275	1	2000	7	0	0	
			2	2000	2	0	0	
			3	2000	4	0	0	
Total			**3**	**6000**	**13**			**13**
5	25	245	1	2000	4	0	0	
			2	2000	6	0	0	
			3	2000	2	0	0	
Total			**3**	**6000**	**12**			**12**

Table 3. Number of micronucleated erythrocytes (MNE) observed in *C. carpio* (period 2).

Animal			Slide No.	Ery. No	Micronucleus type			
No.	L. cm	Wg. gr			A	B	C	Total
1	36	259	1	2000	4	0	0	
			2	2000	8	0	0	
			3	2000	3	0	0	
Total			**3**	**6000**	**15**			**15**
2	27	244	1	2000	5	0	0	
			2	2000	3	0	0	
			3	2000	4	0	0	
Total			**3**	**6000**	**12**			**12**
3	30	251	1	2000	8	0	0	
			2	2000	4	0	0	
			3	2000	6	0	0	
Total			**3**	**6000**	**18**			**18**
4	32	260	1	2000	1	0	0	
			2	2000	3	1	0	
			3	2000	5	0	0	
Total			**3**	**6000**	**9**	**1**		**10**
5	28	235	1	2000	2	0	0	
			2	2000	8	1	0	
			3	2000	2	0	0	
			12	1		13		
Total								

Table 4. Number of micronucleated erythrocytes (MNE) observed in fish *C. carpio* (period 3).

Animal			Slide No.	Ery. No	Micronucleus type			
No.	Length (cm)	Weight (g)			A	B	C	Total
1	20	241	1	2000	8	0	0	
			2	2000	4	2	0	
			3	2000	4	0	0	
Total			**3**	**6000**	**16**	**2**	**0**	**18**
2	25	250	1	2000	2	2	0	
			2	2000	5	1	0	
			3	2000	2	1	0	
Total			**3**	**6000**	***9***	***4***		**13**
3	18	223	1	2000	2	0	0	
			2	2000	5	1	0	
			3	2000	6	1	0	
Total			**3**	**6000**	***13***	***2***		**15**
4	28	256	1	2000	6	1	0	
			2	2000	4	1	0	
			3	2000	3	0	0	
Total			**3**	**6000**	***13***	***2***		**15**
5	15	204	1	2000	3	0	0	
			2	2000	7	1	0	
			3	2000	6	0	0	
Total			**3**	**6000**	***16***	***1***		**17**

Table 5. A brief summary of the whole results observed in *C. carpio* at different period.

Parameter	Periods			
	Control	1	2	3
Gills blood	5.6 ± 1.67	12.4 ± 1.14	13.6 ± 3.0	15.6 ± 1.94
Total MN	28.0	62.0	68.0	78.0
Min. MN	3.0000	11.0	10.0	13.0
Max. MN	7.0000	14.0	18.0	18.0
95% conf	2.0777	1.4157	3.78	2.42

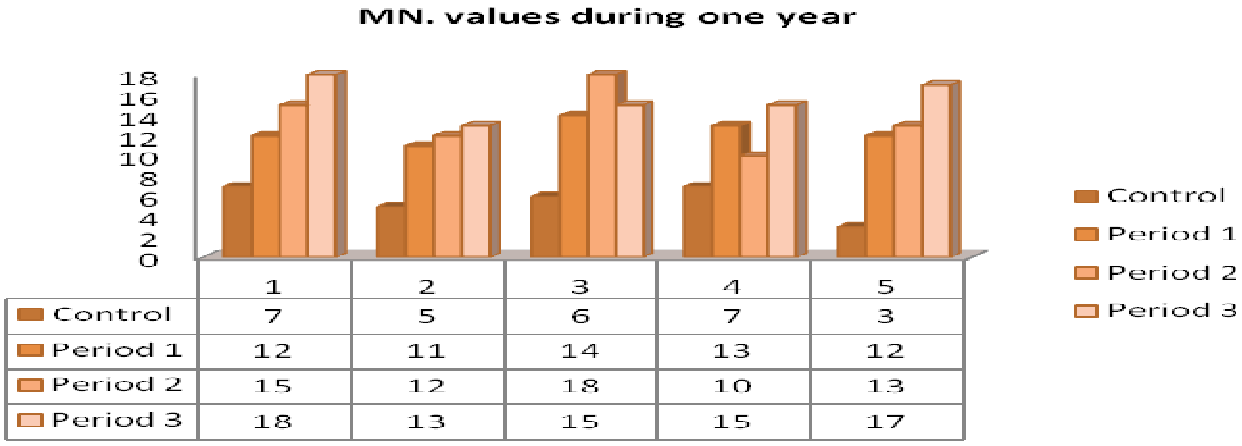

Figure 1. Micronucleus frequencies within red blood cells with standard deviation (n=5).

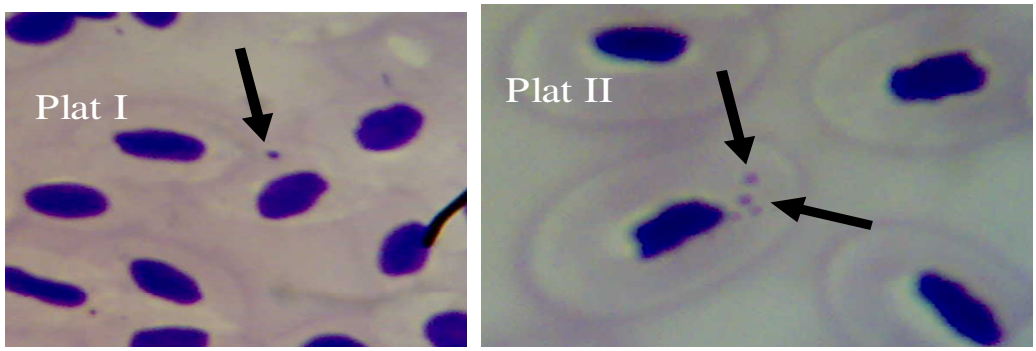

Figure 2. Arrow indicates a micronucleus contained within a red blood cell. Plate I: shows one micronucleus per cell, Plate II: (B) two micronuclei per cell.

related species of *C. carpio* can give a response to genotoxic agents during one year (Tables 2, 3 and 4). This variation of MN frequency may indicate that home waste and other pollutants were increased in periods two and three. Spontaneous MN frequencies (control) were significantly different (f = 21.89, df = 3) while it was not significant between period one and two (f=0.679, df=1), and also between period two and three (f = 1.52, df = 1). It is interesting that the results of the present study showed a significant variation between period one and three (f = 10.03, df = 1) which confirms the hypothesis that this work was built to answer the question; does the water of lake Uluabant contains clastogenic agents and does these agents accumulate by time?

In the light of these observations, the fresh water of Lake Uluabant are continuously contaminated with different pollutants especially clastogenic agents, this may come from the home waste itself or environmental conditions or the interaction between all of these factors.

The increase in the formation of MN indicates that home waste and agricultural pollution can increase the clastogenic effects on peripheral erythrocytes of *C. carpio* 16050 Afr. J. Biotechnol.

and could have similar effects on the human population located around the lake or who consume fishes from this lake.

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