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Clastogenic studies on Tandaha Dam water in Asser

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Abstract

Clastogenic effects of home wastes and agricultural contaminates of Tandaha dam located at Alahad municipality in the southwestern part of Saudi Arabia were investigated in peripheral erythrocytes of *Rana ridibunda*. Examination of blood smears showed that the formation of micronuclei significantly increased during one year ($f=9.89$, $df=3$) with p value < 0.05 and were more abundant compared to the control group. This increase in the formation of micronucleus indicates that agricultural pollution increases the risk of clastogenic effects on peripheral erythrocytes of *Rana ridibunda* and may has similar effects on the human population located around the dam.

Key words: Clastogenic effects, *Rana ridibunda*, environmental genotoxicity, micronucleus test, Tandaha dam, peripheral erythrocytes.

Introduction

The micronucleus test, developed by (Schmid 1975) and (Heddle 1973), is an in vivo and *in vitro* short-time screening method is widely used to detect genotoxic effects (Villarini et al. 1998). It is one of the simplest, reliable, least expensive and rapid screening system for both clastogenic (chromosome breakage and formation of acentric fragments) and aneugenic (chromosome lagging and effects on spindle) effects (Heddle et al. 1983, Orhan et al. 1993).

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Clastogenic and aneugenic agents are known to affect the spindle apparatus, and can be differentiated on the basis of the relative induced micronucleus sizes or in the presence of kinetochores (Heddle et al. 1983, Heddle et al. 1991, Yamamoto and Kikuchi 1980).

In anaphase, any of the chromosome fragments or whole chromosomes which lack a centromere may not be integrated in the nucleus because of 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 the main daughter nucleus and are therefore called micronuclei (Heddle 1973, Schmid 1975).

The advantage of the micronucleus test for mutagenicity screening has been well established in several systems i.e. ovary, bone marrow, epithelial tissues, peripheral blood, liver, exfoliated buccal cells and fetus cells of several laboratory animals or human (Agrawal 1999, Heddle 1990, Konopacka et al. 1998, Krishna et al. 1991, Saleh and Zeytinoglu 2001). Micronuclei formation can occur in any of the dividing cells of tissues of any species (Heddle et al. 1983) as shown by the values of the spontaneous micronucleated erythrocytes (MNE) or in some laboratory animals and mammals (Zuniga et al. 1996). Some organisms such as plants, frogs, birds and fishes were also investigated by micronucleus test to detect the environmental pollution leading to genotoxic damage (Bhalli et al. 2006, Bhunya and Jena 1993, Celikler et al. 2008, Ma et al. 1984, Ma et al. 2005, Zhuleva et al. 1996).

Furthermore, (Hayashi et al. 1998) evaluated the monitoring systems that use aquatic organisms to assess the genotoxicity of water in the field and in the laboratory. In a field study, micronucleus assay was shown to be applicable to micronucleus inducing agents in frogs (Saleh and Zeytinoglu 2001). Aquatic animals have often been used in bioassays to monitor water quality of effluent and surface water (Fernandez and L'Haridon 1992). The development of biological monitoring techniques regarding frogs and fishes offer the possibility of checking water pollution with fast responses on low concentrations of direct acting

toxicants (Al-Sabti 1986, Al-Sabti and Metcalfe 1995, Andrade et al. 2004).

Therefore, it is expected that the contaminants in this dam may accumulate with time leading to decrease the frog counts.

The present study aims at investigating the effect of accumulation of clastogenic factors (agricultural and human waste contaminants) on formation of micronucleus in erythrocytes of peripheral blood of frogs *Rana ridibunda.*

Materials and Methods

Animals: Healthy and actively living frogs *Rana ridibunda* were collected over one year from Tandaha dam near Alahad city (Saudi Arabia) at intervals of four months considering five frogs for each collection. The animals were collected in April 2008. The weight of frogs was ranged from 25-35 g. The same number of animals (15) was also collected from non polluted water to present the control group. Were positive control animals were kept maintained under standard conditions i.e. temperature (27±2) and clean water until they reached the target weight (25-35g) at this stage they were treated by Cyclophosphamide, a well-known genotoxic substance for its reliability for the MN test was used as a positive control for the MN test in amphibian tadpoles (Lajmanovich et al. 2005) The frogs were slaughtered to collect venous blood in heparinized tubes.

Slide preparation and staining: For each frog, three microscopic slides were prepared. Fresh blood samples taken from the venous of each frog within the experimental and control groups collected in heparinized tubes and smeared onto clean slides for. The slides were air dried for 30 min. and then fixed in cold Carnoy fixative for 10 min. After fixation, 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 frogs were used for each of the control, positive control and polluted habitats for each frog, 6.000 cells/frog were analyzed, totaling 30.000 erythrocytes/collection. 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 was used as described by the previous studies: (i) micronuclei should be one-tenth and one-third the 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 indicating variations in shapes and numbers per cell were designated A, B and C to indicate one, two and three micronucleus per cell respectively.

Statistical analysis: The present data was statistically analyzed using one-way ANOVA. The variance was considered significant at %5 level of probability according to Duncan`s Multiple Range test.

Results

The results of the venous blood cells, the total, minimum and maximum. MNE are presented in Table 1. The control animals showed the lowest MNE values when compared with those of the other three groups which significantly increased with time (f= 9.89, df=3).

Table 1. Effect of Tandaha Dam water on total, minimum and maximum MNE and deformed nucleus of *Rana ridibunda* frogs.

Rana ridibunda					
Periods					
Venous Blood	**Control**	**1**	**2**	**3**	**P. con.** Cp
	2.6±0.67	5.4±1.14	8.6±3	10.6±1.94	13.25±1.9
Total MNE	22	30	38	45	58
Min. MNE	3	4	6	8	11
Max. MNE	7	9	18	18	21
Deformed Nucleus	Neg.	Neg.	Neg.	Neg.	Neg.

Min.= Minimum, Max.= Maximum, MNE= Micronucleated Erythrocytes.

The MNE comparisons within the polluted habitats (dam water) revealed no significant differences between groups 1 and 2. Likewise, there were no significant differences (f=0.430, df=1) between groups 2 and 3 but the

differences between the latter and group one were significant (f=6.03, df=1).

The microscopic examination of the micronuclei also showed variations in their shapes and numbers per cell as shown in Figure 1. The micronucleus designated as type (A) were found in all groups, while type (B) micronucleus was found in group 2 Figure 2. The (C) type micronucleus, however, was not found in any group. Deformed shapes of the nucleus was not detected under either dam or control conditions.

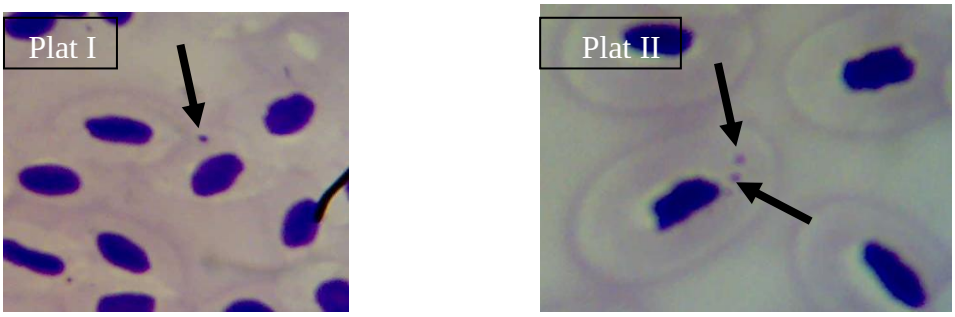

Figure 2. Arrow indicates a micronucleus contained within a red blood cell of *Rana ridibunda*. Plate I: shows one micronucleus per cell, Plate II: (B) tow micronuclei per cell. (C) not detected. Stained by Giemsa and May-grunwald (Sigma). 100X.

Discussion

Bioindicators offer several types of unique information not available from other methods and may be summarized as: (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, the community and the ecosystem; (3) relationships between the individual responses of organisms exposed 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 the remedial efforts in decontaminating waterways (Villela IV 2006). (Al-Sabti and Metcalfe 1995, Marques et al. 2009) and (Saleh and Zeytinoğlu 2001) showed that frogs and fishes are excellent animals 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 also considered appropriate for biomonitoring projects (Lyne 1992) demonstrates that *T. rendalli and O. 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 (Fernandez and L'Haridon 1992, Siboulet 1984). From these studies it may be concluded that frogs can be used for estimating the genotoxic effects of waterborne pollutants.

It is well established that MN can be affected by many factors such as age, sickness, species, feeding, chemical and physical agents and environmental conditions (Al-Sabti 1995, Saleh and Zeytinoglu 2001, Saleh and Sarhan 2007). Thus, to eliminate these factors, healthy, young and active individuals belonging to same frog species namely *Rana ridibunda* were chosen for this experiment.

This experiment clearly demonstrates that *Rana ridibunda* may be considered as an appropriate research material which responds to genotoxic agents within only one year. In terms of MN frequency, the data in table one revealed significant differences between periods 1 and three with a slight increase of period 1 over period 2. These results apparently indicate that the magnitude of accumulation of both home waste and other pollutants in the dam greatly influence the occurrence of high frequency MNE in frogs.

Fortunately, the clastogenic agents accumulated in the dam water at the end of this experiment did not reach the level that causes complete destruction of the whole nucleus.

In the light of these observations, the fresh water of Tandaha dam is continuously contaminated by different pollutants including clastogenic agents.

The buildup of the formation regarding micronucleus indicates that home waste and agricultural pollution can increase the clastogenic effects on peripheral erythrocytes of *Rana ridibunda* and could have similar effects

on human population who either permanently inhabit around the dam area or consume fishes from this dam.

Cyclophosphamide, which is an indirect alkylating agent well-known for its genotoxic properties, was used as a positive control for the MN test in amphibian tadpoles (Lajmanovich et al. 2005) and also in our experiment. Our observation is agreed with the previous studies.

Table 2. The statistical analysis of Tandaha dam water during the periods tested.

Con-period 3	Period1-period2	Period2-period3	Period1-period3
f= 9.89, df=3	f= 0.430, df=1	f= 0.520, df=1	f= 6.03, df=1
P<0.001	P <0.05	P <0.05	P <0.05

Neg.=Negative, MNE= Micronucleated Erythrocytes., Nuc.= Nucleus, Con. = Control, P. con.= Positive control, CP.= Cyclophosphamide.

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