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The Investigation of the Mutagenic Activity of Kars River Sediments on *Orthrias angorae* (Steindachner, 1897) [1]

Oktay ÖZKAN * ✎ Süleyman GÜL** Oya KELEŞ *** Pınar AKSU * Taylan Özgür KAYA **** Gökhan NUR ****

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* Department of Pharmacology, Faculty of Veterinery Medicine, University of Kafkas, Kars - TÜRKİYE
** Department of Biology, Faculty of Science and Arts, University of Kafkas, Kars - TÜRKİYE
*** Department of Pharmacology, Faculty of Veterinery Medicine, University of İstanbul, İstanbul - TÜRKİYE
**** Department of Histology, Faculty of Veterinery Medicine, University of Kafkas, Kars - TÜRKİYE

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Summary

Sediments collected in Kars River were tested for mutagenicity by means of peripheral erythrocyte micronucleus frequency in *Orthrias angorae*. Micronuclei frequencies (MN) of all the groups exposed to the sediments were higher than those of the control group. Statistical analysis showed significant differences between the control and the treatment groups (P<0.001). The MN frequencies of the blood samples in three regions (Selim, Pasacayir, Bogazkoy) on the day 6 have a trend to increase against to control. MN frequencies of samples collected from 36th hours sediment exposure increased in the three districts (Selim, Pasacayir, Bogazkoy) when compared to control groups. On the other hand there is a decrease in only one region (Kars) at 6 days. This study evaluates for the first time the mutagenic load of sediments collected along the Kars River and provides evidence that the presence of genotoxic agents in river sediments correlates with the genotoxic damage (micronucleated erythrocytes) in fish collected from Kars River.

Keywords: *Micronucleus, Mutagenicity, Peripheral erythrocytes, Orthrias angorae, Sediments, Kars River*

Kars Çayı Dip Sedimentinin *Orthrias angorae* (Steindachner, 1897)'da Mutajenik Aktivitesinin Araştırılması

Özet

Sediment numuneleri, *Orthrias angorae*'da peripheral eritrosit mikronukleus testi yoluyla mutajenite testi için Kars çayından elde edildi. Mikronuklei frekansı (MN), kontrol grubuna göre sedimente maruz kalan tüm gruplarda daha yüksek bulundu. Kontrol ve deneme grupları arasındaki fark istatiksel olarak önemli görülmüştür (P<0.001). Üç bölgeden (Selim, Paşaçayır, Boğazköy), sedimente maruz kalan gruplardan 6. günde alınan kan numunelerinin MN frekansları kontrol grubuna göre yüksek bulunmuştur. Sedimente maruz kalan üç bölgeye ait 36. saat numuneleri MN frekansları control grubu ile kıyaslandığında yüksek bulunmuştur. Diğer yandan 6. günde tek bir numune bölgesinde (Kars) bir azalma görülmüştür. Bu çalışma ile Kars çayından elde edilen sedimentin mutajenik yükü ve balıklarda genotoksik hasar (mikronukleili eritrositler) ile sediment içerisindeki genotoksik ajanların varlığına dair kanıtlar ilk kez değerlendirilmiştir.

Anahtar sözcükler: *Mikronukleus, Mutajenite, Periferal eritrosit, Orthrias angorae, Sediment, Kars çayı*

✎ İletişim (Correspondence)
☎ +90 474 242 62 60
✉ oktayozkan@yahoo.com

INTRODUCTION

The toxic wastes and effluents which are deposited into the rivers from the industrial and agricultural enterprices threaten not only the habitat but also humanity [1-4]. The water pollution becomes a serious peril because of the discharge of wastes to the surface waters. It is indicated by the aquatic sources that many industrial wastes and effluents as metal, petroleum refining and chemical sediments are genotoxic [5,6]. Apart from these, the genotoxic activity of the organic compounds in several types of the industrial effluents are serious problem [3,7-9].

Constitute many genotoxic substances merging with the bottom sediments in rivers, lakes and marine coastal regions [3,10]. In every case the sediments will keep the hydrophobic environmental mutagen which will form not only sedimentation process but also accumulation and burial of sediment mutagens [10-12].

While the sediments became contaminated with mutagenic substances, a certain hazard may occur to indigenous biota [3,13]. This type of contamination then causes DNA adducts micronuclei, chromosomal aberration and cancer. One of the techniques which is commonly carried out to identify genomic alteration in animals is the micronucleus test [3,6,14-16]. This test is more beneficial because it can be performed on interphase to any proliferating cell population irrespective of its karyotype [3,17,18].

The province of Kars is located in the region of North Eastern Anatolia in Turkey, and is on the Historic Silk Road. It is a famous city with its historical and archaeological ruins, natural beauties and rich folklore culture.

Kars River runs from the Allah-akhbar Mountains to the Arpacay dam lake and is approximately 93 km in length. It is a typical freshwater ecosystem of great importance in regard to biodiversity and to aesthetic value. Due to its morphological characteristics, the Kars River is suitable habitat for a variety of species, especially fish for both recreational and intensive amateur fishing.

The Kars River flowing through the Kars province has been polluted by the Cement factory, local wastes and agricultural chemicals.

The *O. angorae* which is fostered by the bottom sediment is susceptible to the bottom sediment pollutants in a high degree. The selected stations are the right places where the pollution is clearly observed.

The aim of this study is to determine the mutagenic activities of the river sediment against the peripheral blood of the *O. angorae* as measured the MN frequency.

MATERIAL and METHODS

This study was performed with 50 adult *O. angorae* specimens which were captured by electrofishing from Kura-Aras river basin (Lat 38° C 30' E, long 44° 84' N) in Bolukbasi region. Fish were transferred alive to the laboratory where the experiments were carried out. Animals were kept in aquaria under laboratory conditions (20°C and natural photoperiod) for a 7 day acclimatization period. The physiochemical characteristics of the water are listed in *Table 1*.

Table 1. *Physiochemical properties of the water used in the aquarium*

Tablo 1. *Tanklarda kullanılan suyun fizikokimyasal özellikleri*

Parameter	Value
Temperature (°C)	19±1
pH	7.2-7.3
Dissolved oxygen (mg/L)	8-9
Total hardness (CaCO3) (mg/L)	150-170

Sediments were sampled in four different sites of Kura-Aras river basin. The selections of sample stations were determined by taking the flowing style of the Kars River into consideration as it is illustrated in *Figure 1*. The first station is the town called Selim (Lat 38° C 31' E, long 44° 81' N). The second station is the locality called Pasacayir (Lat 38° C 33' E, long 44° 95' N) which is just before the entrance of Kars city, the third station is the city Centrum (Lat 38° C 34' E, long 44° 97' N), and the last one is the village called Bogazkoy (Lat 38° C 34' E, long 45° 01' N) in the exit of the city.

The experiments were conducted under normal light/night illumination during June July 2006. Fishes were not fed during the experimentation period. The sediment was collected by means of sedimentation instrument. The sediment samples were immediately brought to the laboratory in the closed polyethylene tanks.

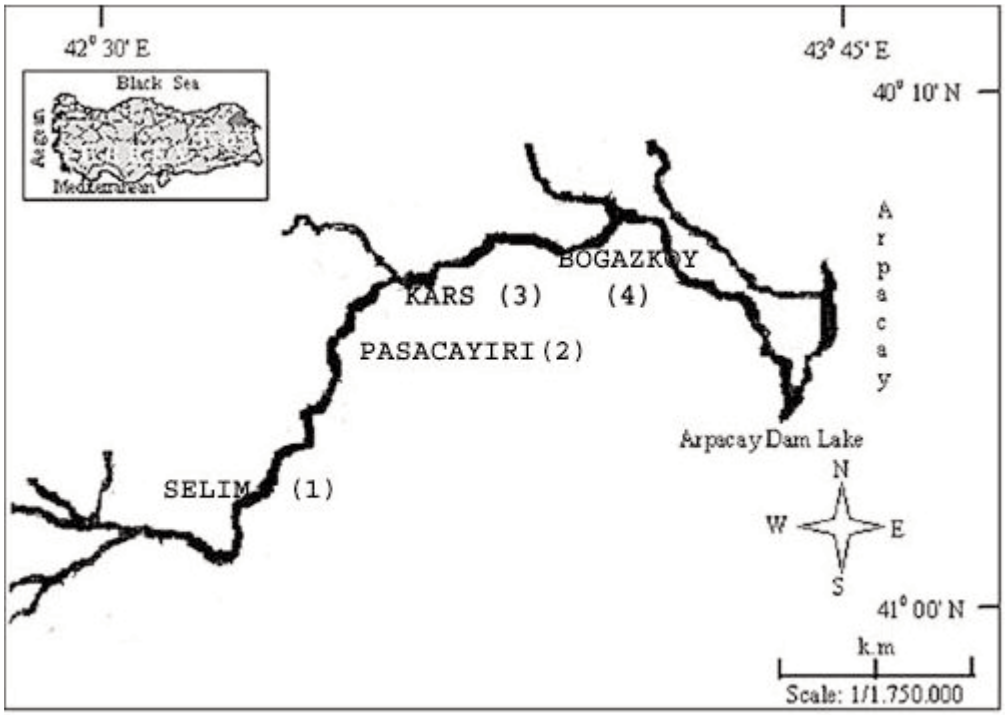

Fig 1. Map indicating the locals of the four reaches along the Kars River where the sediments were collected

Şekil 1. Kars çayı boyunca sediment numunelerinin alındığı dört bölgenin harita üzerinde görünüşü

The laboratory study was done according to the methods of Vigano et al. by the aim of the direct exposures to the sediments samples of the fish [19].

The study was planned as four treatment and one control groups. Each group contained ten fishes and 30 litters of water. The fishes kept hungry, and the tanks were aerated and the water was circulated during the treatment period. Sediment samples were calculated as 4.6 kg for each tank [19].

The fish were kept in these conditions for six days. The experiments were replicated twice under the same condition. Slides were examined under a light microscope (x 100). From each fish, five slides were prepared, and from each slide 1000 cells were scored. Only no-refractory particles with the same colour of erythrocytes nuclei were interpreted as micronuclei [20].

For statistical analysis, one way analysis of variance (ANNOVA) was used to compare the groups.

RESULTS

The 36th hours micronuclei results of the samples in the peripheral blood of the fish are summarized in *Figure 2.* Micronuclei frequencies of all the sediment groups were higher than the control. Statistical analysis showed significant differences among control and Kars ($P<0.001$) and Bogazkoy ($P<0.001$) treatment groups.

The MN frequencies of the blood samples in the day 6 have a trend to increase in three regions (Selim, Pasacayir, Bogazkoy) when compared to control. On the other hand there is a decrease in only one region (Kars) *(Figure 2)*. MN frequency in sixth day peripheral blood samples is shown in *Figure 2*. MN frequencies of samples collected from sixth day sediment exposure increased in the three districts (Selim, Pasacayir, Bogazkoy) when compared to control groups. On the other hand a decrease was indicated only in the one district (Kars).

There were no any significant differences between control and treatment groups when the results were analysed statistically. However, there were significant differences between Selim and Kars treatment groups ($P<0.05$).

Comparative results of MN frequencies of control and treatment groups after 36 h and day 6 are shown in *Figure 2*.

While the MN frequencies of three treatment groups (Kars, Pasacayiri, Bogazkoy) were decreasing, other two treatment groups were increasing.

When the values of the groups in 36th hours

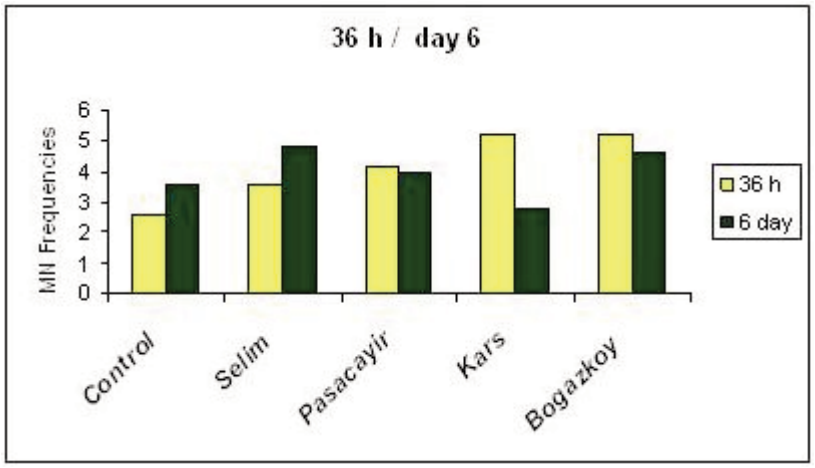

Fig 2. The evaluated results compared with 36 hours and 6th days MN frequency and control groups

Şekil 2. Kontrol grubu ile 36. saat ve 6.gün MN frekans sonuçlarının karşılıklı değerlendirilmesi

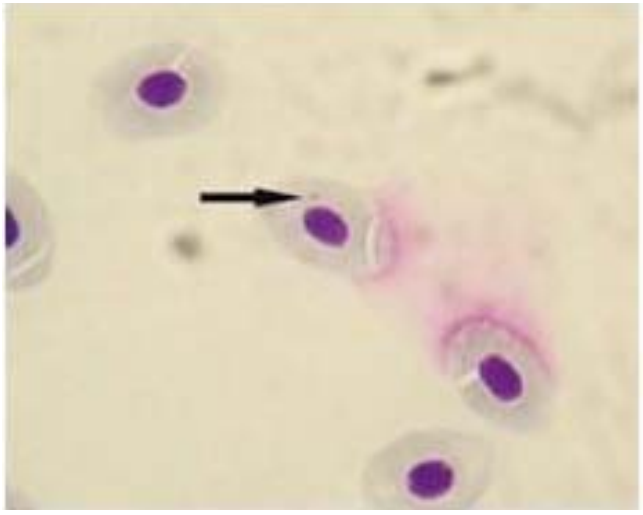

Fig 3. Erythrocytes of *O. angorae*. Arrow: A micronucleated erythrocyte (magnification 1000X)

Şekil 3. *O. angorae* eritrositi. Ok: Mikronukleili bir eritrosit (büyütme X 1000)

and 6th day were compared, it has been found an increase in control and decrease in Kars treatment group.

DISCUSSION

Thousands of synthetic chemical compounds have been stored in most part of the countries to be exploited in industry [3,13], and the production of such chemicals have been annually increased. During the process of these chemicals, not only the atmosphere but land, streams, rivers, lakes and seas have continually been polluted. 80.000 of chemicals have been indicated in commerce and the proportion of mutagens among chemicals is estimated nearly 20%. Both the xenobiotics and carcinogens released into the surroundings migrate into surface waters and gather in sediments. Xenobiotics ever existing in water or sediments are observed by the gills, the skin, or gastrointestinal tract of fish or epidermal cells or root hairs in plants inhabiting chemically polluted aquatic environment [3]. From the standpoint of genetic hazard to humans and aquatic ecological significance, the determination of the potency and quantification of mutagens /carcinogens in surface waters are one of the significant issues [3,8,12,13]. It is observed in some cases that each chemical usually consists of low levels that are very hard to indicate in surface waters [16]. Meanwhile, various bioassays which are sensitive to genotoxicants have been used as an integral instrument in the evaluation of the risk of surface waters as complex mixtures and are present to aid in the identification of chemicals posing a genetic hazard to human health and aquatic organisms [3,13,16]. Micronuclei can be analysed in diverse fish cell sorts such as gill cells, kidney erythrocytes and hepatic cells. However, the main advantage of the use of peripheral blood cells (erythrocytes or lymphocytes) is that annihilating the animal is avoided, which allows the consecutive monitoring of the some population [14].

Kars is a province where the industrial affairs are less than agricultural affairs. The cement factory is only the main sources polluting the environments. But apart from the pollution caused by the agricultural affairs the urban wastages also form as serious problem.

Kars River flows through the city of Kars so it is prone to every sort of pollution. In the sediment sampling stations along the river, there has not been any refining plant or any preventive operations. These mentioned studies evaluating the mutagenic activity regarding Kars River have not been take in

to consideration previously. Meanwhile, in an observation performed about heavy metal level, there is no any risk over the allowed values [21]. The evaluation of the mutagenic activity of sediment complex gives us a chance to get more adequate information more than the chemical test in detail. For these purpose Ames test and in-vivo MN tests become more significant although it is possible to use many genotoxicity tests [10,17-19,22].

When the chemical mixture in the sediment complex is taken into consideration in-vivo MN test is much more important with respect to evaluation in the genotoxicity [3,13,14]. The present study evaluated for the first time the mutagenic load of sediments collected along the Kars River. MN can occur as consequences of both structural and numerical chromosomal aberrations, the frequencies of micronucleated erythrocytes in control groups exposed to sediments were analysed. Micronucleus induction was observed in both fish groups and the results are presented in *Figure 2*. The maximum response for micronucleus induction by sediments was observed at 6 day after exposure. Maria et al. reported an increase in MN frequency in Anquilla anquilla exposed to the sediment samples obtained from an area where paper industry is extensive [12]. Vagas et al. demonstrated that the organic sedimental extracts belonging to the area that detected heavy metal and DDT showed frameshift mutational activity in the microscreen page induction test and Salmonella microsome test [19,22].

Viganò et al. detected frameshift mutation from in vitro tests of sediment samples taken from the Po river and DNA adduct formation was reported in the liver of the fishes in the region [19]. In that study, they also observed an increase in MN frequency for the peripheral blood samples of the fishes [22]. Minisi et al. reported that sediment taken from the river Tiber in Italy exhibited a mutagenic activity in *Vicia Faba* [23]. Reichert et al. showed that mutagenic phenomena, affected by polycyclic aromatic compounds inside the sediment complex and genotoxic contaminants, were interrelated with neoplastic disease in the livers of the fishes [25]. Tagliari et al. detected a mutagenic activity by *Salmonella microsuspension* test for the organic sediment extract taken from the rivers Cadeia and Feitoria in Brasil. Regarding sediment chemical analysis, important data are obtained from the results of genotoxicity evaluations. Evaluations considering sediment as a whole are used widespread [6]. Baumann reported that there is a strong cause-effect relationship between neoplastic disease seen in the fish population in the nature and genotoxic agents arising from the environmental pollution, in water and sediment [11]. Jha et al. reported that genotoxic activity in *Mytilus edulis* in embryo-larval period was found positive for the sea sediment samples collected from Brixham harbor in England [8].

In this study, for *O. angorae* species fed with sediment, detection of the mutagenic activity of the complex formed inside the sediment was aimed rather than the chemical explanation of the contamination. MN frequency values of the blood samples taken in the 36th hour showed that river contamination is increasing at the Kars city center and city exit and acute genotoxicity effect is observed. These results indicate a contamination originated especially from the urban wastes. However, on the 6th day of sediment exposure, MN frequency values obtained from blood samples were not statistically significant. This situation may result from the half life of the compounds that contaminates the region, fishes metabolic functions, or interaction of chemicals inside the sediment. The ones that did not sediment in control group were kept in disinfected water. As 36th hour and 6th day MN frequency values valid for this group were compared, statistically significant increase in these values were detected. This observation might indicate that chemical substances used for disinfection in the feeder water in the city may have mutagenic effect. In the disinfection process of the drinking water, sodium hypochlorite (NaClO), chlorine dioxide (ClO_2) or per acetic acid (CH_3COO_2H, PAA) are widely used and there are studies related to mutagenic activities of the water disinfectants. Gustavino et al. showed genotoxic effect of water disinfectants, sodium hypochlorite, chlorine dioxide, in Cyprinus carpio 5 and Bolognesi et al. used comet test and MN test to determine genotoxic effect of the disinfectants in zebra muscle cells [24].

In conclusion, the present study evaluated for the first time the mutagenic load of sediments collected along the Kars River. This study provided evidence that the presence of genotoxic agents in river sediments correlates with the genotoxic damage (micronucleated erythrocytes) in fish collected from Kars River.

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