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The genotoxic and biochemical effects of wastewater samples from a fat plant in Erzurum

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Abstract

*Toxicological effects of effluent exposure may be acute or chronic, and can occur at all levels of biological organization, from the molecular to the ecosystem level. The most common strategy to investigate potential adverse effects and toxicological modes of action following effluent exposure is the use of biological tests. The frequencies of sister chromatid exchanges (SCEs) and superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) enzyme activities were assessed on human blood cultures exposed to wastewater samples (WWS). The water samples were taken from a local fat plant in Ilıca region (Erzurum). Metal ions (Fe, Cu, Mn, Zn, Pb, Cd) were measured by an Atomic Absorption Spectrophotometer (AAS). According to our results, SCE rates increased significantly (P<0.0.5) in dose-responded manner after treatments with WWS. And there was a significant positive correlation between SCE frequencies and WWS concentrations (*r *= 0.98). On the other hand, SOD, CAT and GSH-Px enzyme activities decreased in erythrocytes. These results reveal that high level of heavy metal content (especially lead and cadmium) of WWS caused genotoxic damage by oxidative stress. Thus, this could lead to adverse health effects for exposed human and animal populations.*

Key Words: *Genotoxicity, oxidative stress, human blood, wastewater, fat plant.*

Erzurum'daki bir yağ fabrikasından alınan atıksu örneklerinin genotoksik ve biyokimyasal etkileri

Özet

*Atık maruziyetinin toksikolojik etkileri akut ya da kronik olabilir ve moleküler düzeyden ekosistem düzeyine kadar her biyolojik organizasyonun tüm aşamasında ortaya çıkabilir. Atık maruziyetini takiben bu atığın toksikolojik etki yolunu ve olumsuz etkilerini araştırmanın en yaygın stratejisi biyolojik testler kullanmaktır. Kardeş kromozom değişim frekansları (SCEs) ve süperoksit dismutaz (SOD) katalaz (CAT) ve glutatyon peroksidaz (GSH-Px) enzim aktiviteleri atık su örneklerine (WWS) maruz kalmış insan kanı kültürlerinde araştırıldı. Su örnekleri Ilıca (Erzurum) yöresinde bulunan bir yağ fabrikasından alındı. Metal iyonları (Fe, Cu, Mn, Zn, Pb, Cd) Atomik Absorpsiyon Spektrofotometresi (AAS) ile ölçüldü. Sonuçlarımıza göre WWS uygulamasından sonra SCE frekansı önemli ölçüde yükseldi (P<0.0.5). Ayrıca SCE frekansı ile WWS konsantrasyonları arasında önemli bir pozitif korelasyonun olduğu görüldü (*r *= 0.98). Öte yandan eritrositlerdeki SOD, CAT ve GSH-PX enzim aktiviteleri de azaldı. Bu sonuçlar WWS içindeki yüksek seviyedeki metal iyonlarının (özellikle kurşun ve kadmiyum) oksidatif stres vasıtasıyla genotoksik hasara neden olduğunu göstermektedir. Bu sonuçlara göre, ortaya çıkan bu hasarın atık suya maruz kalan insan ve hayvan populasyonlarında olumsuz sağlık etkilerine sebep olabilir.*

Anahtar kelimeler: *Genotoksisite, oksidatif stres, insan kanı, atıksu, yağ fabrikası.*

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1. Introduction

The chemicals including heavy metals and organic compounds present in wastes are assimilated by aquatic species, pass through the food chain, and bioaccumulate upon long-term exposure [1]. Moreover, environmental mutagens including heavy metals may be an important risk factor for human health [2, 3]. Besides the direct health effects, they may be mutagenic or carcinogenic and lead to several human afflictions like cancer and cardiovascular diseases [4]. The mechanism of metal carcinogenicity remains largely unknown, although several lines of experimental evidence suggest that a genotoxic effect may be involved [5, 6]. Determination of the chemical composition and the genotoxic potential of wastewaters is crucial for environmental protection and public health [7]. At this point biological tests, especially short-period bioassays can detect a wide range of substances that can cause genetic damage and enable quantification of mutagenic hazard even when there are no sufficient data about identity and physico-chemical properties of compounds present in wastewater [8, 9]. As a matter of fact, SCEs are included as genotoxic endpoints to reflect DNA damage or biomarkers of exposure. Similarly, the activities of SOD, CAT, and GSH-Px are recently used to monitor the development and extent of damage due to oxidative stress [10].

Local fat plant (Erzurum) works as a butter-factor during all summer season and wastages are drained into the Karasu River without filtering process. In a previous study, the effluent of this factory induced embryotoxicity in the zebrafish due to its heavy metal content [11]. But, according to our knowledge, no investigations were carried out for evaluating the genetic and oxidative effects of WWS of this plant. Thus, it was aimed to elucidate the genetic and oxidative effects induced by different concentrations (0.5, 1, 2, 5, 10 and 20%) of WWS in present study. For this aim, the genotoxic potential was assessed using SCE test and the activities of main antioxidant enzymes such as SOD, CAT and GSH-Px were also determined for evaluating oxidative effects.

2. Material and methods

2.1. Water sampling and analysis

WWS were taken from a fat plant in Erzurum, TURKEY in summer season of 2007. The samples were from the exit of a small-scale wastewater treatment facility using biological oxidation with active sludge. The wastewater is a combination of sewage wastewater and discharges from different processes in the industry. Metal ions (Fe, Cu, Mn, Zn, Pb, Cd) were measured by an Atomic Absorption Spectrophotometer (Perkin-Elmer) [12]. No other chemical measurements have been done (Table 1).

Table. 1. Water quality parameters and chemical characteristics of fat plant (FP) effluent and control.

Sample	pH	TDS (mg/l)	CON (µS/cm)	DO (mg/l)	Cu (µg/L)	Fe (µg/L)	Cd (µg/L)	Pb (µg/L)	Mn (µg/L)	Zn (µg/L)
FP	7.96	535	844	2.10	2.50	66.7	76.6	73.63	47.52	5.1
Control	7.05	645	994	5.80	nd	nd	nd	nd	nd	nd

All data were mean data of two replicates for each treatment. nd; showed less than determined limit.
DO, TDS, and CON showed dissolved oxygen, total dissolved solids and conductivity respectively.

2.2. In vitro treatments

The collected WWS were sterilized using a milipore filter (0.22 μm) and added to the cultures at final concentrations of 0.5, 1, 2, 5, 10 and 20%. Human blood was obtained by veinpuncture from three non-smoking donors. After supplementations of WWS, the blood was incubated for 1 h at 37 ˚C to adjust body conditions, except for testing SCE (see below). The whole blood culture with physiological water studied as a control group.

2.2.1. SCE assay

Cultures were set up according to a slight modification of the protocol described by Evans and O'Riordan (1975) [13]. A 0.5 ml aliquot of heparinized blood was cultured in 5 ml of culture medium (Chromosome Medium B, Biochrom®). WWS were added to the culture tubes just before incubation (72 h). The control samples were incubated without water samples. With the aim of providing successive visualization of SCEs, 5-bromo-2'-deoxyuridine (BrdU) (Sigma®) was added after culture initation. Exactly 70 h and 30 min after beginning of incubations, demecolcine (Sigma®) was added to the medium. After hypotonic treatment (0.075 M KCl), three repetitive cycles of fixation in methanol/acetic acid solution (3:1, v/v), centrifugation, and then stained by use of the fluorescence plus Giemsa (FPG) technique for the inspection of SCE rate. For each treatment condition, well-spreaded 25 second division metaphases were scored, and the values obtained were calculated as SCEs per cell.

2.2.2. Enzyme activities

Erythrocytes were obtained from heparinised blood samples by centrifugation (3000 rpm, for 20 min) at 4 ˚C. SOD activity was determined by the method of Misra and Fridovich (1972) [14], which is based on the ability of superoxide dismutase to inhibit the process of epinephrine self-oxidation in alkaline medium. SOD activity was measured by monitoring the increase in absorbance at 480 nm. CAT was determined by the method of Aebi (1984) [15]. 5 μL of a catalase solution was added to 3 ml H_2O_2 (54 nm H_2O_2 in 50 mM phosphate buffer, pH 7.0), and the depletion in H_2O_2 was measured spectrophotometrically (Beckman DU 500, USA) at 240 nm, at 25 ˚C for 60 s. GSH-Px activity was measured using hydrogen peroxide as substrate [16]. Potassium azide was added to inhibit CAT. Conversion of NADPH was monitored continuously in spectrophotometer at 340 nm for 3 min at 25 °C.

2.3. Statistical analyses

Statistical analyses were performed using SPSS 12.0 software. The two-tailed Student's t-test was used to compare the mean values obtained between treated and control groups. Correlation was assessed by calculating the Pearson correlation coefficient (*r*). Statistical decisions were made with significance level of 0.05.

3. Results

The mean SCE rates after treatments with various concentrations (%) of WWS are presented in Table 2. The results showed that the samples at the concentrations of 2, 5 and 10% but not 0.5 and 1% caused statistically significant increase in SCE frequency. There was a significant positive correlation between SCE frequencies and WWS concentrations ($r = 0.98$; $P<0.05$). However, after the treatment with the concentration of 20% of the samples, the cultures found to be sterile.

Table 2. The effects of WWS on the number of SCEs in human peripheral lymphocytes.

Treatments	No of examples	Mean SCE value ± S.D
Control	3	6.74 ± 1.74
0.5	3	6.87 ± 1.66
1	3	7.06 ± 1.75
2	3	7.94 ± 1.96*
5	3	13.43 ± 2.36*
10	3	17.25 ± 3.55*
20	3	-

* means statistically different from the controls at the level of P<0.05. Values are expressed as mean ± SD for three cultures in each group.

The results of the biochemical experiments are presented in Figures 1, 2 and 3. The statistically significant decreases of SOD, CAT and GSH-Px enzyme activities were found after the application of the WWS to the cultures.

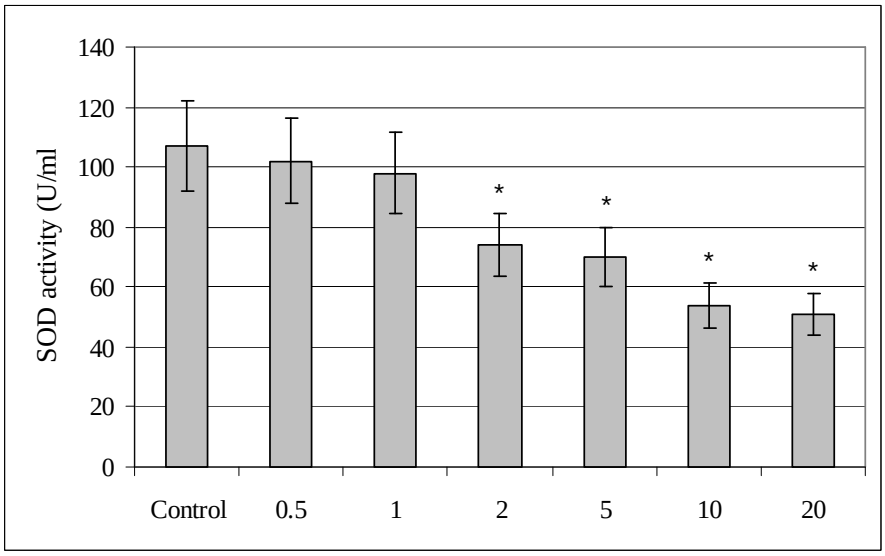

Figure 1. The activity of SOD in WWS treated cultures. * means P <0.05.

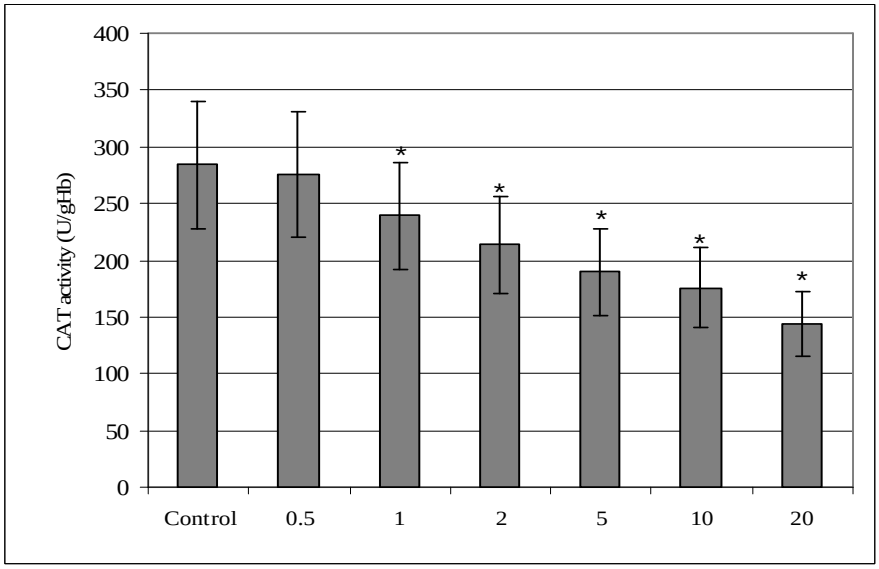

Figure 2. The activity of CAT in WWS treated cultures. * means P <0.05.

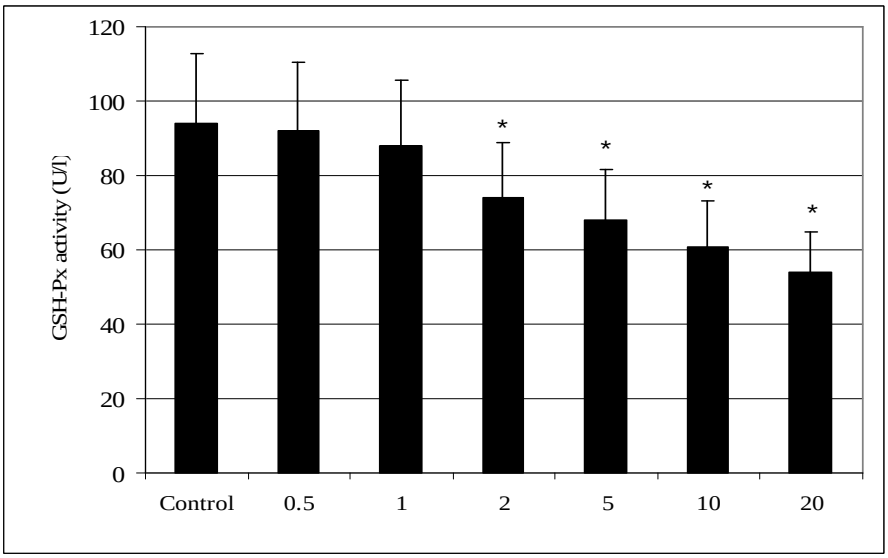

Figure 3. The activity of GSH-Px in WWS treated cultures. * means P <0.05.

4. Discussion

The results of the present study showed that the concentrations of Pb and Cd metal ions in WWS were higher than the other metals. We also established that the samples caused to the development of oxidative stress and genotoxicity. In fact, heavy metals common element used for beneficial and commercial purposes but with a potential to cause environmental pollution and causing deleterious effects affecting human health [17]. Many possible cellular mechanisms have been hypothesized to explain the metal toxicity, but oxidative stress is a major process responsible for triggering excytotoxicity pathways that led to membrane peroxidation and generation of reactive oxygen species (ROS) [18]. The decreases of main antioxidant enzyme activities such as SOD, CAT

and GSH-Px after treatments with WWS were observed in this study could be explained by the presence of high levels of Pb and Cd ions. Khan et al. (2008) [19] found that Pb induced oxidative stress in exposed human populations. The similar finding was also reported for Cd [20]. Moreover, interactions between metal ions such as Fe, Cd, Ni, Cr or Cu could effect the generation of 8-OHdG and the formation of DNA strand breaks and demonstrated that these lesions could arise by different mechanisms [21]. Nagy et al. (2005) [22] have reported that increasing oxidative stress caused DNA damage. Indeed, the increase in malondealdehyde (MDA) as due to heavy metal treatments is an important sign of oxidative status and elevated lipid peroxidation in a variety of lipid systems, such as plasma, organs and cell membranes [23, 24].

In a previous study, it was reported that the WWS containing a large amount of metals like Cd, Cu, Cr and Zn, caused several tissue damages (renal and hepatic) in laboratory rats. And this effect seemed in part to be mediated by suppression of antioxidant system [25]. The utility of antioxidant enzymes, such as SOD, CAT and GSH-Px, as biomarkers of heavy metal pollution in WWS were investigated using the *Allium cepa* (onion) system. The WWS, containing the amounts of heavy metals equivalent to those found in the wastewater, resulted in steep declines in antioxidant enzyme activities in *A. cepa* [26]. Again Labrot et al. [27] were found the relationships between heavy metal exposure, lipid peroxidation and some enzyme activities in a mollusc (*Cohicula sp*.), an earthworm (*Eisenia fetida*) and a fish (*Brachydanio redo*). Similar findings were also obtained by the present study performed on human blood cultures.

On the other hand, it was reported that membrane damages evoke active gene expression and cell proliferation [28]. So SCE occurs spontaneously in proliferating cells and is regarded as a manifestation of damage to the genome. Thus the higher levels of metals in culture medium could cause increased rate of SCEs after exposure to WWS as compared to controls in the present study. In support of our findings, various *in vitro* and *in vivo* studies have shown the ability of WWS to induce genetic damage. Note in this context that, the heavy metal content of WWS were analysed in the *A. cepa* genotoxicity test and found to induce significant chromosome aberrations [29]. The application of prokaryotic tests systems with biomarkers such as DNA fragmentation in different tissues of test organisms seemed to be a useful combination for the assessment of cytotoxic and genotoxic potential in surface waters and secondary effluents [30]. The mutagenicity of wastewater was determined by the Ames Salmonella/microsome test and found to exhibit mutagenic activity in *Salmonella typhimurium* TA 98 and TA 100 [31]. In addition, heavy metal concentrations in WWS from the industrial estate were determined and a significant decrease in the survival of DNA repair defective *Escherichia coli* mutants *recA, lex A*, and *polA* was observed as compared to their wild-type counterparts in the presence of WWS [32]. The analysis of micronuclei (MN) (genotoxicity endpoint) in peripheral blood of rainbow trout (*Oncorhynchus mykiss*) after exposure to WWS exhibited a significant increase of MN in exposed *O. mykiss* specimens compared to control fish [33]. In a recent investigation, Krishnamurthi and his collegues [34] used four genotoxicity assays namely chromosomal aberration, DNA strand break, DNA laddering and P53 accumulation tests in human peripheral mononuclear blood cells and showed genotoxic potentials of contaminated WWS.

In conclusion, WWS from a local fat plant in Erzurum produced oxidative stress and DNA damage in human peripheral blood cultures. The increased SCE frequency in lymphocytes and decreased enzyme activities in erythrocytes in blood cultures indicate

potential hazards. And the exposure of waste water could lead to adverse health effects for exposed human and animal populations.

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