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The evaluation of the genotoxic and oxidative damage potentials of *Ulothrix tenuissima* (Kütz.) *in vitro*

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Abstract

Several alga species are known to produce a variety of toxic metabolites that pose a threat to aquatic organisms, animals and humans. Moreover, these metabolites have been thought to cause serious diseases including certain cancers and neurodegenerative disorders. On the other hand, *Ulothrix* is a genus of filamentous green algae, generally found in fresh water and marine and abundantly available in some lakes and rivers of Turkey. To our best knowledge, no study has been performed to assess the genotoxic and biochemical effects of *U. tenuissima* on cultured human blood cells. Therefore, in order to determine clastogenic or aneugenic effects of aqueous alga extracts the micronucleus assay was carried out. Nuclear division index (NDI) in peripheral lymphocytes was also analyzed for cytotoxicity evaluations. In addition, biochemical parameters (total antioxidant capacity (TAC) and total oxidative stress (TOS)) were examined to determine oxidative effects. For this aim, we obtained heparinized blood samples from three healthy persons. The alga samples were collected from Porsuk Pond in Hasankale (Erzurum, Turkey) in summer period of the year 2010. The aqueous extracts of this species were added to cultures at different concentrations (0 to 5000 ppm) for 72 h. Our results showed that this alga did not cause any statistically important changes in the rates of studied genotoxicity endpoint. But dose-dependent alterations were observed in TAC and TOS levels and NDI rates. In conclusion, *U. tenuissima* was found to be non-genotoxic but caused sterility at higher concentrations due to oxidative stress.

Keywords

Algae, lymphocytes, genotoxicity, oxidative status, *Ulothrix tenuissima*, whole blood culture

Introduction

Algae are unusual forms of plants that thrive on water systems and rely on the process of photosynthesis for growth. Because of their biologically active compounds, many algae species are being used for various economic purposes including soil conditioner, treatment for acid in drinking waters and animal food additives (Johansen, 1981). Until now, it was reported that more than 2400 natural products have been isolated from seaweeds of subtropical and tropical populations (Manilal et al., 2009). Recent findings revealed that seaweeds exhibited antiviral, antibacterial, antifungal and antitumoral potentials in relation with their chemical content (Zandi et al., 2010). On the other hand, over the past three decades, the frequency and global distribution of toxic algal incidents appeared to have increased, and human intoxications from novel algal sources occurred. And several algae species are reported to produce potent toxins that impact human health through the consumption of contaminated shellfish and finfish and through water or aerosol exposure (Klemencic and Griessler-Bulc 2010; Van Dolah, 2000). As a matter

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of fact, genetic (Falconer and Humpage, 2005; Sayer et al., 2005) neuronal (Bonislawska et al., 2003; Wang, 2008), and hepatic (Masango et al., 2008) damages occured after exposure to these toxins produced by algae. Falconer and Humpage (2006) indicated that there was a need for epidemiological studies on adverse health effects in exposed populations to clarify the extent of the health risk.

The genus *Ulothrix* is the largest group of the green algae and consists of 11 species including the type species, *Ulothrix tenuissima* (Lokhorst, 1985). This genus generally found in fresh and marine water. Its cells are normally as broad as they are long, and they thrive in the low temperatures of spring and winter. They become attached to surfaces by a modified holdfast cell. The algae species belonging to genus *Ulothrix* are abundantly available in some lakes and rivers of Turkey (Tuzen et al., 2009). And it is revealed that algae may be easily accessible sources of natural drugs that could be used as a possible food supplement or in the pharmaceutical industry after their safety evaluations. At this point, published information on *Ulothrix* species and the related human health effects is very scarce. Therefore, in this investigation, we aimed to describe whether the cytogenetic and oxidative effects of *U. tenuissima* aqueous extracts on cultured human blood cells can be utilized as a possible food supplement and within the pharmaceutical industry. With this aim, not only micronucleus (MN) frequencies but also nuclear division index (NDI) rates have been established as related to the dose of aqueous extracts of the algae on human lymphocytes. In addition, important oxidative parameters, total antioxidant capacity (TAC) and total oxidative stress (TOS) were used to monitor its antioxidant or pro-oxidant activities *in vitro*.

Materials and methods

Experimental design

Whole heparinized human blood from three healthy non-smoking donors between the ages 26 and 28 with no history of exposure to any genotoxic agent was used in our experiments. Questionnaires were obtained for each blood donor to evaluate exposure history, and in addition, informed consent forms were signed by each donor. In all the volunteers involved in this study, hematological and biochemical parameters were analyzed and no pathology was detected. A various concentrations (0, 1, 5, 10, 25, 50, 100, 200, 250, 500, 1000, 2000, 2500 and 5000 mg/L) of aqueous algae extracts were tested in blood cultures. MN and NDI rates were assessed in peripheral lymphocytes. Experiments conformed to the guidelines of the World Medical Assembly (Declaration of Helsinki). The cultures without extracts were studied as control group.

MN assay

Human peripheral blood lymphocyte cultures were set up according to a slight modification of the protocol described by Evans and O'Riordan (1975). A 0.5 mL aliquot of heparinized blood was cultured in 6 mL of culture medium (Chromosome Medium B; Biochrom, Berlin) with 5 mg/mL of phytohemagglutinin (Biochrom). The MN test was performed by adding cytochalasin B (Sigma; final concentration of 6 μg/mL) after 44 h of culture. At the end of the 72-h incubation period, the lymphocytes were fixed with ice-cold methanol:acetic acid (1:1). The fixed cells were put directly on slides using a cytospin and stained with Giemsa. All slides were coded before scoring. The criteria for scoring micronuclei were as described by Fenech (1993). At least 1000 binucleated lymphocytes were examined per concentration for the presence of one, two or more micronuclei.

NDI analysis

For cell cycle analysis, 400 cells per treatment group were scored for the presence of one, two or more than two nuclei and the nuclear division index (NDI) was calculated as follows:

$$\mathrm{NDI} = [1\mathrm{N} + (2 \times 2\mathrm{N}) + (4\times > 2\mathrm{N})]/\mathrm{C}$$

where 1N is number of cells with one nucleus, 2N—with two nuclei, and >2N—with more than two nuclei, C—number of cells examined (Konopacka and Rogolinski, 2004).

TAC and TOS analysis

TAC and TOS assays were carried out by commercially available kits (Rel Assay Diagnostics®, Turkey) in plasma samples obtained from blood cultures for 2 h (Erel, 2004).

Sampling and identification

Algae were collected from Kuzgun Dam Reservoir in the Eastern part of Turkey in summer period of the year 2010. Samples were dried at room temperature for 48 h.

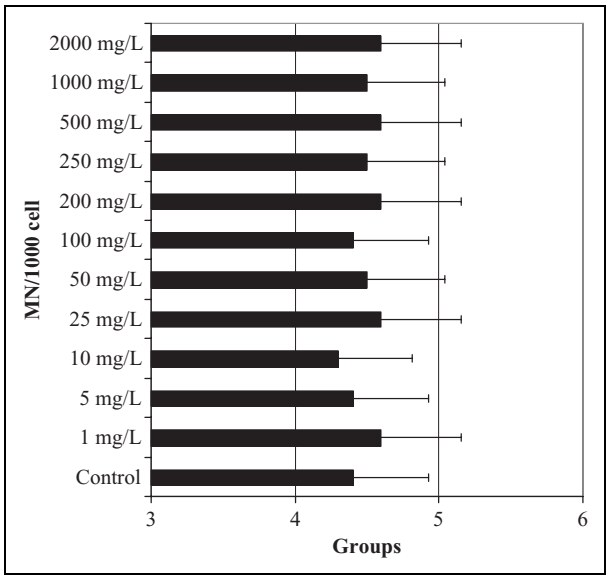

Figure 1. Rates of MN in cultured human lymphocytes treated with *U. tenuissima* water extracts for 72 h.

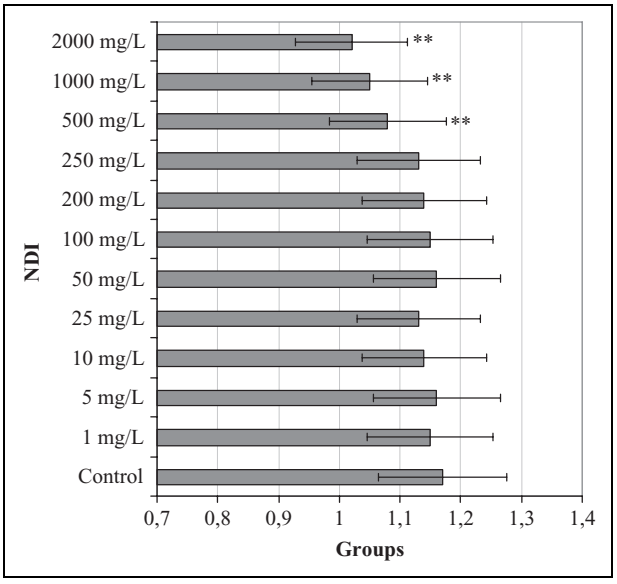

Figure 2. Rates of NDI in cultured human lymphocytes treated with *U. tenuissima* water extracts for 72 h.

The taxonomic identifications were confirmed by H. Gurbuz. The voucher specimen (*U. tenuissima*—KKEF—161020) have been deposited at the herbarium of Kazim Karabekir Education Faculty, Atatürk University, Erzurum, Turkey.

Extraction

For water extraction of air-dried and powdered algae, 20 g sample was mixed with 400 mL distillated and boiling water using magnetic stirrer for 15 min. Then the extracts were filtered over Whatmann No. 1 paper.

Statistical analysis

Statistical analysis was performed using SPSS software (version 13.0, SPSS, Chicago, IL, USA). The Duncan's was used to determine whether any treatment significantly differed from controls or each other. Statistical decisions were made with a significance level of 0.05.

Results

The effects of algal extracts on DNA damage were determined by MN assay in human lymphocytes. Our results indicated the algal extracts did not show any mutagenic effects at tested concentrations (Figure 1). However, these extracts at 500, 1000 and 2000 mg/L reduced the rates of NDI in human lymphocytes *in vitro* (Figure 2). And, the cultures found to be sterile at concentrations of 2500 and 5000 mg/L. The obtained results from the control and experimental cultures for TAC and TOS levels are represented in Figure 3. Significant

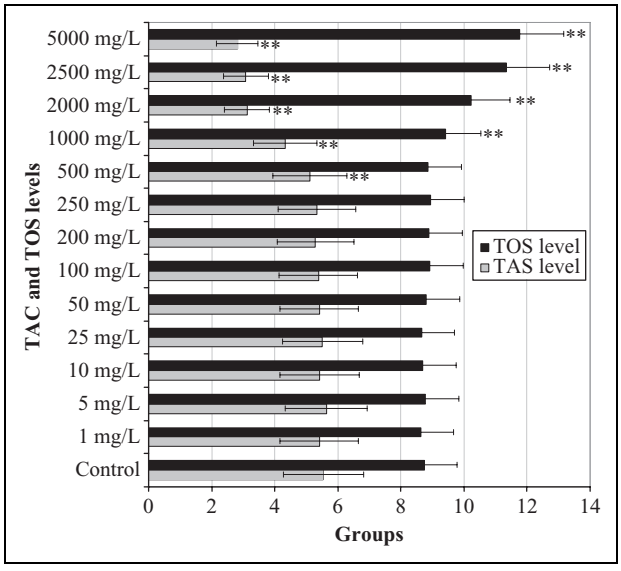

Figure 3. The TAC (mmol Trolox equiv./L) and TOS (μmol H_2O_2 equiv./L) levels in cultured human lymphocytes treated with *U. tenuissima* water extracts for 2 h.

reductions of plasma TAC levels were found in algae extracts-treated group (at concentrations above than 250 mg/L) when compared with the control ($p < 0.05$). Likewise, these extracts caused significant alterations in plasma TOS (at concentrations above than 500 mg/L) due to applied concentrations.

Discussion

In the present study, it was established that the extracts of *U. tenuissima* species were non-genotoxic for the first

time. Because, the results obtained by us did not indicate any significant increases in the ratios of MNs in lymphocytes exposed to algae extracts as compared to control values. In fact, MN assay provides a measure of both chromosome breakage and chromosome loss or non-disjunction in clastogenic and aneugenic events, respectively (Karaman et al., 2009; Sisman and Türkez, 2010). The damaged DNA can lead to aneuploidy and/or chromosomal instability, which is believed to be a major contributor to tumor progression (Erol, 2010). In accordance with our findings, the extracts of *Porphyra tenera* was found to be non-mutagenic. Furthermore, this alga showed a suppressive effect on mutagen-induced umu C gene expression in *Salmonella typhimurium* TA 1535/pSK 1002 strain (Okai et al., 1996). Similar effects were also reported for the algae *Sargassum latifolium* (Gamal-Eldeen et al., 2009). Again non-mutagenic effect of *Haematococcus pluvialis* algae was detected by using a mutagenicity test with *Salmonella typhimurium* strains TA100, TA1535, TA98, TA1537, TA1538 and E.coli WP2 uvr A (Cyanotech Corp., 1999). On the contrary, the presence of the genotoxicity of algal extracts (*Polysiphonia fucoides*) was shown in erythrocytes of rainbow trout (*Oncorhynchus mykiss*) by Comet assay and MN test (Del Barga et al., 2006).

The results of the present study also indicated that the aqueous extracts of *U. tenuissima* caused sterility of human blood cultures for 72 h at concentrations of 2500 and 5000 mg/L. In addition dose-dependent alterations were observed in both oxidative parameters (TAC and TOS levels) and NDI rates. Hence, we can suggest that *U. tenuissima* may have cytotoxics effect on human blood cells *in vitro*. The previous experimental evidences indicated that oxidative damage was involved in algal extracts induced toxicity (Ding et al., 1998). At this point, the cytotoxic effects of this green algae could be attributed to oxidative stress-induced by algal contents. As a matter of fact, *Karlodinium micrum* was considered cytotoxic and embryotoxic algae due to its fractions with hemolytic activity (Zhou et al., 2011). Similarly, the cytotoxic potentials of the crude extracts of four green marine algae (*Cladophora rupestris*, *Codium fragile* ssp. *tomentosoides, Ulva intestinalis* and *Ulva lactuca*) were also determined (Spavieri et al., 2010). Again, the extracts of several green algae species such as *Cladophoropsis vaucheriaeformis*, *Sargassum swartzii, Cystoseira myrica* and *Colpomenia sinuosa* were consedered as anticancer agents due to their cytotoxic potentials (Harada and Kamei, 1998; Khanavi et al., 2010).

Finally, our results clearly indicated that the water extracts of *U. tenuissima* collected from Erzurum, Turkey had no mutagenic and antioxidant effects on human lymphocytes. But, the extracts caused sterility of cultures due to oxidative stress at higher concentrations. It is concluded that *U. tenuissima* can be consumed or used in cosmetics and pharmaceuticals safely, but it is necessary to take into consideration the cytotoxicity at increasing doses. We also offer that this *in vitro* approach which includes the collaborative use of cytogenetic endpoinds (MNs) and oxidative parameteres (TAC and TOS levels) will serve to compare the potential health risks of algal extracts related to mutagenesis or carcinogenesis.

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