TETHYS ENVIRONMENTAL SCIENCE
an International Journal

Potential Genotoxicological Evaluation of Ulexite on *Daphnia magna*

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Research Article

Citation: Ergenler, A., (2024). Potential Genotoxicological Evaluation of Ulexite on *Daphnia magna*. *Tethys Env. Sci.* 1(1): 27-32.

DOI: 10.5281/zenodo.10878376

Received: 08 January 2024

Accepted: 27 February 2024

Published: 25 March 2024

Abstract

Numerous industries depend on boron (B) compounds, such as the glass industry, the agricultural sector, the cosmetics industry, and the pharmaceutical industry. There are a number of significant borate minerals found in nature, including borax (BX), colemanite, and ulexite. In this study, the effect of ulexite, a commercially important clay mineral, on DNA damage parameters in freshwater cladocerans *Daphnia magna* were investigated. For this purpose, *Daphnia magna* were exposed to different doses of ulexite (35; 40 and 45 mg/L) in a static test apparatus for 96 hours according to OECD guideline 211. Toward the completion of the investigation, the Comet assay was used to assess the DNA damage frequency (%), Arbitrary unit (%), and Genetic damage index (%) in *D. magna*. The results indicated that ulexite low toxicity DNA damage at all tested concentrations. As a results, Ulexite could be used low toxicity in aquatic environments at the doses studied.

Keywords: *Ulexite, Daphnia magna, DNA Damage, Comet Assay*

Introduction

The chemicals containing boron (B), particularly boric acid and borax, find widespread use in a variety of industries, including food production, beauty products, medicines, and glassware items (Ince et al., 2010). According to the findings of previous research, boron substances have beneficial determined to wellness on the health of animals. The advantages include an enhancement in the overall well-being and the bolstering of the immune system. Boron compounds is a commonly occurring boron mineral in the natural environment, colemanite and ulexite are other noteworthy examples of borate minerals. Ulexite a compound consisting of sodium, calcium, and hydroborate, has been detected at atypical areas inside the The crust of the earth.The presence of borates such as, boron compounds in animal and human diet has the ability to have substantial effects on several metabolic and physiological processes inside the body. However, a comprehensive understanding of

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the biochemical process behind boron (B) and its contained compounds remains incomplete. Several assumptions are necessary to understand the impact of vitamin B on hormones, processes including enzymes, cell membrane functions (Ince et al., 2010; Alak et al., 2018; 2019a; 2019b; 2019c, 2020). These assumptions are based on a number of different data sources. According to Akyıldız (2012), ulexite is a hydroxide that originates from boron minerals. It has a more delicate structure and an amorphous appear, which is characterized by accumulation. Hardness measured on the mohs scale, density ranging from 1.95 to 2.95 grams per cubic centimeter Boron oxide makes up around 43 percent of the 2.5 and brilliant oxide, according to DPT (2001).

In particular, the presence of this occurrence may be seen in some parts of the Balıkesir, Kütahya, and Eskişehir rivers located in Turkey (DPT, 2001). There is a lack of data on the genotoxic outcomes of various bor chemicals in animals. Consequently goal of this research was to investigate the impact that ulexite had on *Daphnia magna*.

Material and Methods

Daphnia magna was used in the experiment completed at the Aquaculture Research and Development Center, located within the Faculty of Marine Sciences and Technology at Iskenderun Technical University in Turkey. We performed treatment tests on D. magna in accordance with OECD guideline 211 (OECD, 2012) to examine and contrast the impacts of ulexite. In summary, a total of twenty neonates, each less than 24 hours old, were placed in 100 mL glass beakers. These of ulexite were made as assets and subjected to continuous stirring throughout the transfer to the test vessels. The experiments were carried out under a 16:8 hour light:dark cycle at a temperature of 20±1°C. To minimize evaporation, the beakers were covered with watch glasses. The selection of control and three distinct doses of ulexite (35, 40, and 45 mg/L) was based on previously identified concentrations in the aquatic environment, which were used to conduct an acute test for a period of 96 hours. A supplementary set of positive controls, consisting of Ethylmethane sulfonate at a concentration of 5.0 mg/L, which is often associated with genotoxic chemicals, was created for a duration of 96 hours. A total of three replicates of eighty daphnids were included in each treatment group. At the conclusion of the trial, the specimens were exclusively subjected to manipulation only for the purpose of conducting the Comet test. The DNA damage in samples was assessed using the alkaline comet test, following the procedures outlined by Mayer et al. (2002). Cavalcante et al. (2008) adapted the cellular dissociation technique, whereas Singh et al. (1988) conducted single cell gel electrophoresis. The slides underwent neutralization, applied ethidium bromide staining, and were then quantified using an image analysis system. The classification of nucleoids into five classes (0-4) was determined by evaluating the intensity of the comet tail. For the sake of comparison, the damage percentage, arbitrary unit values, and genetic damage index were computed. The intensity of the comet's tail in the obtained samples was measured by an example consisting of 2000 cells. The statistical measures used in this study were presented as the mean ± the standard deviation of the mean (SD). The statistical normality of the data was assessed using the Kolmogorov-Smirnov test, while the homogeneity of variance was assessed using Levene's test. The researchers conducted a one-way analysis of variance (ANOVA). The mean values were compared using Duncan's test.

Results and Discussion

The results of 35 mg/L, 40 mg/L and 45 mg/L of ulexite applied groups as compared to the untreated control fish and positive were presented in Table 1. The table describes the mean as well as the standard deviations of the DNA damage frequency (%), arbitrary units values (AU), and genetic damage index (%) observed in *Daphnia magna*.

Table1. Means and standard deviations of DNA damage on the *Daphnia magna* obtained from the control and three different concentrations of ulexite.

Groups (mg/L)	Damage Frequency (%)	Arbitrary Unit (AU)	Genetic Damage Index (GDI) (%)
Positive Control	96.66 ± 0.57^{d}	378.00 ± 4.08^{c}	3.78 ± 0.48^{c}
Negative Control	24.66 ± 0.58^{a}	64.66 ± 3.78^{a}	$0,64 \pm 0.03^{a}$
35	28.33 ± 2.08^{b}	67.66 ± 5.85^{a}	0.67 ± 0.05^{a}
40	38.00 ± 2.00^{c}	106.00 ± 6.92^{b}	1.06 ± 0.06^{b}
45	39.00 ± 1.00^{c}	106.00 ± 4.58^{b}	1.06 ± 0.04^{b}
P	***	***	***

The data are shown as arithmetic mean ± standard deviation. Values with different superscripts in each column indicate significant differences. p indicates significance level between DNA Damage obtained from control and three different concentrations of ulexite effect on *Daphnia magna* (*, p<0.001).

The control group daphiads had the lowest damage frequencies (%) at 24.66±0.58, whereas the positive control group had the greatest DNA damage (99.35±1.07%), as expected. This group was statistically distinct from all other groups (P<0.001). The observed frequency of DNA damage in the Ulexite treatment groups varied between 28.33±2.08% and 39.00±1.00%. The results of the statistical analysis indicated a statistically significant increase (P<0.001) in the control group when compared to the other treatment groups. Table 1 presents comparable statistical findings according to the Arbitrary Unitve Genetic damage index values observed in the Ulexite treatment groups. Furthermore, the groups treated with ulexite at concentrations of 40 and 45 mg/L did not exhibit any significant genotoxic effects. The groups exhibited a statistically significant disparity, with the toxicity level being around 60 percent lower compared to the mutagenic group, which was used as the positive control group.

Discussion

Ulexite minerals, composed of sodium, calcium, and hydroborate, is found at random locations inside the Earth's crust. Ulexite has significant prominence as the primary boron mineral used in the synthesis of boron compounds. The reaction mechanism of boron and its components remains poorly understood. However, several investigations in the literature have shown that hypoxide does not induce harm to DNA damage pathways. (Alak et al., 2021). The findings of this research suggest is an accurate illustration of the evaluation of DNA damage caused by low toxicological levels following use by aquatic species.

The examination of DNA damage levels was carried out in this work by using the comet assay test technique, as described by Ergenler and Turan (2023). In their study, Alak et al. (2021) evaluated the oxidative parameters of the mechanism of action of ulexite in the zebrafish brain. They exposed zebrafish to a dose of ulexite (5, 10, 20 and 40 mg/l) for four days. Multiple biochemical analyzes were conducted on oxidative responses in brain tissues. As a result, they determined that the effect of low ulexite concentrations on the antioxidants system (< 40 mg/l) did not cause oxidative stress in the antioxidant system in the zebrafish brain. In addition to other doses, they found that low ulexite concentrations did not cause DNA damage or apoptosis.Other studies tahat Alak et al. (2020) in their study, they determined low dose UX (5, 10 and 20mg/L) with the Micronucleus test. In their examination, they found that they did not find any damage. Additionally, in another literature study, Turkez et al. (2012) determined in their investigate that due to genoprotective effect of borax, the increase in micronucleus frequency decreased dramatically. In addition, it can be said that boron and its derivatives generally inhibit some pro-oxidant enzymes and effectively reduce tissue damage and DNA damage (Pawa and Ali, 2006). They also assumed that ulexite could be used safely in aquatic environments. By addition to protecting DNA with lipids from the oxidization, UX and associated chemicals have positive effects for eight-OHdG concentrations with caspase-3 activity (Yazıcı et al., 2014; Alak et al., 2020).

In conclusion, the literatures are consistent with our study that ulexite has low toxicity on *Daphnia magna*. Since ulexite tends to be low toxicity, it can be used in relevant biomedical or other sectoral applications.

Acknowledgment

Thanks to the Scientific &Technological Research Council of Turkey (TUBITAK-BİÇABA) program for A. Ergenler.

Conflict of Interest

The author declares that she has no competing interests.

Author Contributions

A.E. performed all the experiments and drafted the main manuscript text. In addition to collected samples and performed analysis.

Ethical Approval Statements

Local Ethics Committee Approval was not obtained because experimental animals were not used in this study.

Data Availability Statement

The data that supports the findings of this study are available on request from the author. The data are not publicly available due to privacy or ethical restrictions

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