ISSN: 2458-8989

Natural and Engineering Sciences

NESciences, 2022, 7(2): 190-199 doi: 10.28978/nesciences.1159286

Monitoring DNA Damage in Suez Pufferfish (*Lagocephalus suezensis*) from the Northeastern Mediterranean

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Abstract

Industrial effluents, agricultural runoff, and municipal wastewaters contain unknown substances and complex mixtures that are released into the environment and can lead to contamination of surface and subsurface waters. Such activities have endangered the existence of ecosystems and their inhabitants. Changes in the genome caused by genotoxic agents led to mutations and pose a burden to the populations of fish species. In the present study, we have used the alkaline Comet assay to detect the genotoxicity in Suez pufferfish (*Lagocephalus suezensis*) sampled from two different gulfs (Iskenderun and Mersin Bay), Northeastern Mediterranean. At the end of the study, the damage frequency (%), arbitrary unit and genetic damage index (%) were evaluated in gill and liver cells of *L. suezensis* by comet assay. The DNA damage in the gill and liver cells of *L. suezensis* in the present study were observed with a higher level of DNA damage in gill cells compared to liver cells in both the Iskenderun and Mersin Bays. The highest level of DNA damage (55.01±1.02%), arbitrary unit (143.01±7.21) and genetic damage index (1.43±0.07 %) were found in gill cells of *L. suezensis* from Mersin Bay. Statistically significant differences were found between DNA damage, Arbitrary unit and genetic damage index values of the two locations ($p < 0.001$). In conclusion, this study indicates that comet assay gives sensitive results in monitoring the pollution, especially the pollution of the gulf, and thus it might be used as a standard method in regularly monitoring the pollution of the coastal ecosystem.

Keywords:
Lagocephalus suezensis, DNA damage, comet assay, Iskenderun Bay, Mersin Bay

Article history:
Received 02 April 2022, Accepted 25 July 2022, Available online 08 August 2022

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Introduction

Genotoxicity is a word in genetics defined as a destructive effect on a cell's genetic material affecting its integrity. Genotoxic substances are known to be potentially mutagenic or carcinogenic when inhaled, ingested or penetrate the skin. As many mutations may ultimately result in cancer (or be part of the multistep process of carcinogenesis), mutagens are typically also carcinogens. Genotoxins can cause three primary effects on organisms by putting an impact on their genetic information. Genotoxins can be carcinogens (cancer-causing agents), mutagens (mutation-causing agents) or teratogens (birth defect-causing agents). In most of cases, genotoxicity leads to mutations in various cells and other body systems. DNA damage in a somatic cell may result in a somatic mutation, which may lead to malignant transformation (cancer). For genotoxicity assessment many in-vitro and in vivo tests have been developed that, with a range of endpoints, detect DNA damage or its biological consequences in prokaryotic (bacterial) or eukaryotic (fishes, mammals, yeast or birds) cells (Ternjej et al., 2010).

Contamination of water resources by genotoxic compounds is a global issue. Although aquatic ecosystems are equipped with a variety of physical, chemical and biological mechanisms to eliminate or reduce the adverse effects of toxic substances, toxicants may cause changes in development, growth, reproduction and behaviour, or even death in freshwater organisms. The test has been applied to a wide variety of aquatic species including fish, molluscs, crustaceans, and worms. Among these aquatic organisms, fish provide excellent material for the study of the genotoxic or carcinogenic potential of water samples, due to their ability to metabolize, concentrate and store waterborne pollutants (Ateeq et al., 2005; Kaur et al., 2018).

The pufferfish that belongs to the Tetraodontidae family consists of approximately 120 species, of which seven are found in the Mediterranean Sea. Six are in this area of which four are Lessepsian migrants (Turan et al., 2017). With the opening of the Suez Canal, the lessepsian species quickly spread into the Mediterranean (Ergüden et al., 2013; Turan et al., 2014; Gürlek et al., 2016; Doğdu et al., 2016; Stamouli et al., 2017; Turan, 2020). For the first time, these species have spread to a wide area in the seas around Turkey since first being noted in the early 2000s (Turan et al., 2018). Subsequently, this Lessepsian invasive species have established large populations along the coasts of many countries of the eastern basin such as Israel, Lebanon, Turkey, Cyprus and Greece, while still rapidly expanding westwards along the coasts of Egypt, Libya, and along the entire Tunisian coastline (Turan et al., 2017). Pufferfishes are highly invasive species in the Mediterranean (Doğdu et al., 2021).

However, a Comet assay based on the Suez pufferfish cells has not yet been found in the literature research. In the present study, we firstly investigated the genotoxicity in Suez pufferfish (*Lagocephalus suezensis*) as a highly invasive species, sampled from two different gulfs (Iskenderun and Mersin Bay), Northeastern Mediterranean.

Materials and Method

Sample Collection and Preparation

Suez pufferfish (*Lagocephalus suezensis*) were collected from Iskenderun Bay (36°40’13-36°05’59) and Mersin Bay (37°23’02- 35°11’05), in the north-eastern Mediterranean Sea, Turkey during January-February 2022 by trawler net (Figure 1; Figure 2).

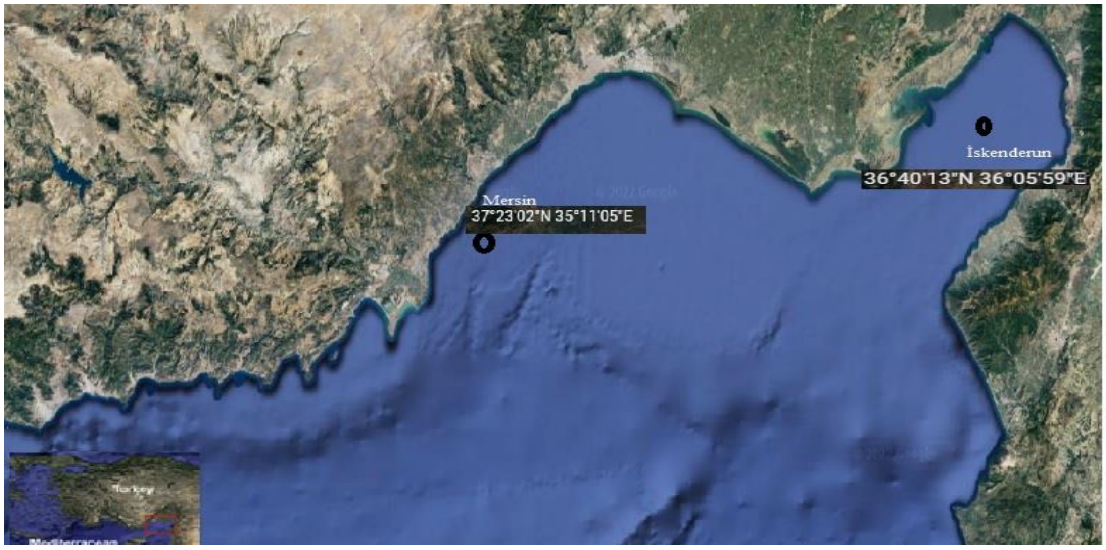

Figure 1. Location of the study area

Figure 2. Lagocephalus suezensis sampled from in the North-eastern Mediterranean Sea

In totally, ten specimens of Suez pufferfish (*Lagocephalus suezensis*) were sampled from two different bays (Iskenderun and Mersin Bay), Northeastern Mediterranean and the samples were immediately transported to the laboratory on ice in an insulated box. All the specimens were measured to the nearest cm, whereas weights were recorded with the use of electronic balance to the nearest 0.01 g, individually (Table 1). The Suez pufferfish samples were euthanized for gill and liver removal and immediately after dissection, they were carefully washed with phosphate buffer. Throughout this study, all chemicals used were analytical grade.

Table 1. Length-weight of *Lagocephalus suezensis* from in the North-eastern Mediterranean Sea, Turkey

Species	N	Size range (cm)	Weight range (g)	Locations
L. suezensis	10	15.00±2.03	84.33±3.75	Iskenderun Bay
L. suezensis	10	12.90±1.57	72.36±1.08	Mersin Bay

Comet Assay

Alkaline comet assay formation of DNA damage was assayed by the alkaline comet assay essentially as described by Mayer et al., (2002). The cellular dissociation method modified from Cavalcante et al. (2008) was used in the comet assay. The test for sensitivity of the comet assay as used in this study is described below and indicated that DNA damage caused by gamma irradiation of 0.2 Gy is clearly detectable. Gill and liver tissues of Suez pufferfish were homogenized to get single-cell suspension and centrifuged at 3000 rpm at 4 °C for 5 min for the cell suspension, and then the cell pellet was retained. Cell viability was evaluated by the Trypan blue exclusion test (Anderson et al., 1994). Singh et al. (1988) were followed for performing the single cell gel electrophoresis. The slides were neutralized with ice-cold 0.4 M Tris buffer (pH 7.5) and stained with 80 ml ethidium bromide (20 mg/mL) and counted with an image analysis system. Images of 100 cells from each sample (gill and liver cell) were visually scored as proposed by classifying the nucleoids, which were assigned to one of five classes (0–4; with 0 signifying no visible tail and 4 almost all DNA in the tail) according to intensity of the comet tail. For comparison of the data from the comet assay, the damage percentage (%DF), the arbitrary unit values (AU) and genetic damage ındex (GDI) was calculated as defined by Pitarque et al. (1999) and Collins (2004).

Statistical analysis

The intensity of comet tail in the samples obtained from Iskenderun and Mersin Bay was calculated based on 2000 cells. All values were given as mean ± standard deviation of the mean (SD). One-way analysis of variance (ANOVA) was used for statistical evaluations of data. All statistical analyses were performed by IBM SPSS Statistics 21 software.

Results and Discussion

The DNA damage parameters as damage frequency (%), arbitrary unit (AU) and damage index (%) are given in Table 2.

Table 2. DNA damage in the gill and liver cells of pufferfish from Iskenderun Bay and Mersin Bay was analyzed by comet assay. (*, P<0.05; **, P<0.01; ***P<0.001).

GILL	**Iskenderun Bay**	**Mersin Bay**
Damage Frequency (%) ***	44.67±0.57	55.01±1.01
Arbitrary Unit (AU)**	115.67±8.02	143.01±7.21
Damage Index (DI) (%) **	1.15±0.08	1.43±0.07
LIVER		
Damage Frequency (%) *	36.01±4.01	43.01±1.73
Arbitrary Unit (AU) *	84.01±10.81	99.33±4.51
Damage Index (DI) (%)	0.84±0.10	0.99±0.04

A higher level of DNA damage in gill cells compared to liver cells was observed in Suez pufferfish samples from both Iskenderun bay and Mersin Bay (Table 2). The highest level of DNA damage as damage frequency (%), arbitrary unit (AU) and damage index (%) in gill cells were 55.01±1.01%, 143.01±7.21, 1.43±0.07% at the pufferfish samples from Mersin Bay, respectively (Table 2). Likewise, the highest level of DNA damage as damage frequency (%), arbitrary unit (AU) and damage index (%) in liver cells were 8.995±1.118%, 0.885±0.005µm, 3.349±0.264TMi at the pufferfish samples form Mersin Bay, respectively. Significant differences (P<0.01) in DNA damage especially in gill cells were found between the pufferfish samples from Mersin Bay and Iskenderun Bay (Table 2).

The present study attempted to determine whether genotoxic potential existed in the coastal ecosystem of Iskenderun and Mersin Bays using a comet assay at the Suez pufferfish (*L. suezensis*). Also, this research is a preliminary study showing the environmental hazards of potential pollutants found in both Bays although the type and amount of these pollutants remain unknown. Based on the results of DNA damage on gill cells and liver cells from the pufferfish in both bays, it was determined that the mutagenic potential of Mersin Bay was more than relatively Iskenderun Bay, besides the difference between the relatively Mersin Bay and Iskenderun Bay was statistically significant (P< 0.005). Because pollution values in water and fish could not be checked at the date and locations of the study, we are not sure about the situation of the pollution status of the Iskenderun and Mersin bays. Thus, results from the fish were evaluated to make comparisons with those from the study with the different bay.

In our previous study, we reported medium DNA damage of gill and liver cells in Trachinotus ovatus from Mersin Bay using comet analysis to determine the quantity of DNA damage (Turan & Ergenler, 2019). Ameur et al., (2012) observed a significant increase in DNA damage (as % tail DNA) 89.23% and 93.97% respectively in mullet and sea bass from Bizerte Lagoon, Tunisia. Similarly, Slobodskova et al. (2019) reported that chronic pollution of the aquatic environment causes destructive changes to DNA in gill and digestive gland cells of *C. grayanus* from Nakhodka Bay, Sea of Japan (East Sea). Also, the adverse consequences of pollution

identified based on the highly sensitive and relevant methods for determining genotoxicity (including those used in our work such as the DNA comet assay), were reported from Hong Kong Bay (Siu et al., 2008), the Vostok Bay (Sea of Japan) (Kukla et al., 2022), waters off the western coast of India (Sarker et al., 2018). DNA damage is of high concern as it can lead to changes at the gene level resulting in mutagenicity or carcinogenicity in fish tissues owing to chronic exposure to heavy metals or PAH etc. in the marine ecosystem (Udroiu, 2006; Turan & Ergenler, 2019).

Comet Assay or the single-cell gel (SCG) with fish also have shown potential as an in situ biomonitoring tool for detecting genotoxic agents in the marine environment (Kaur et al., 2018, Turan & Ergenler, 2022). This technique is a more useful approach for assessing DNA damage, and more advantageous as it detects low levels of DNA damage, requires only a very small number of cells, is cheaper than many techniques, is easy to execute, and quickly displays results (Kaur et al., 2018; Bolognesi et al., 2019). Many studies have employed the alkaline comet assay with fish species for monitoring DNA damage in field studies, in both freshwater and marine environments (Bolognesi et al., 2019; Turan et al., 2020a;2020b; Cui et al., 2021).

Mersin Bay was found partially polluted by mutagenic and genotoxic compounds than Iskenderun Bay, and the current study indicates that Comet assay in the fish yields sensitive results in monitoring pollution; especially the gulfs pollution and thus, it might be used as a standard method in regularly monitoring the pollution of the coastal ecosystem. Furthermore, Suez pufferfish (*L. suezensis*) as the new biological model may be the potential to be widely used in biomonitoring in the marine ecosystem.

Acknowledgements

Thanks to The Scientific &Technological Research Council of Turkey (TUBITAK-2211/C National PhD Scholarship Program for Priority Areas) and The Council of Higher Education for 100/2000 PhD scholarship program for A. Ergenler. Abstract of this study was presented at 2th International Symposium on Pufferfish/Lionfish held on 20-22 May 2022 in Turkey

Author Contributions

F.T., A.E and F.B. performed all the experiments and drafted the main manuscript text.

Conflict of Interest

The authors declared that no conflict of interest.

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