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Micronucleus Test Good Biomarker for Determination of Genetic Changes in Aquatic Organism

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Abstract

Micronucleus experiments are mutagenicity-testing systems used to identify chemicals and pollutants that cause DNA particles to change, such as micronuclei in the cytoplasm of Interphase cells. Damage caused by genotoxic pollutants on DNA is the first effect that occurs in aquatic organisms. This paper reported that the micronucleus test gives sensible results in monitoring the chemical and anthropogenic pollution.

Keywords: Micronuclei test; Aquatic pollution; Genotoxicity

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Introduction

Today, both natural phenomena and the environmental impacts of human activities are a fact and the development of new methods to minimize the harmful effects on biological resources, ecosystems and human health frightens the eyes of the environment makers and regulators [1]. It is present in many polluted surface waters that threaten the survival of organisms, disrupt physiology, or cause carcinogenesis. The effects of mutations induced by these pollutants may continue stagnant for a few generations or may have a large impact on the population pool. For this reason, the use of biomarkers has increased in order to identify the effects of contaminants at the genomic level [1-3]. Chemical substances that are toxic to genes called as 'genotoxic'. Toxicity of DNA molecules in the genes and toxic agents (genotoxins) resulting from interaction with the next generations is known as 'genotoxicity'. Damage caused by genotoxic pollutants on DNA is the beginning to occur in aquatic organisms and therefore they used in widely the genotoxicity studies. Despite the presence of many harmful substances in water and sediments, which harmful contaminants accumulated by living organisms and trigger DNA or cell damage, affect the ecosystem [4]. Determination the concentration of such substances in the tissues analytically with present chemical methods is not possible, not economic and time consuming. For this reason, biological methods based on carcinogenic and mutagenic substance screening have become important in the tissues of indicator organisms. For this purpose, ecotoxicological studies have gained intensity to genotoxic substances cause degradation of DNA structure or DNA breakage. Monitoring of toxicants causing deterioration of DNA structure gives strong ideas for toxicological investigations [1-3].

In ecotoxicological, studies have intensified in recent years in order to determine the impacts of pollutants on the gene structure of living things. The genotoxicity of contaminated waters was good explored using standard *in vitro* genotoxicity assays [5]. In addition, aquatic species affected by the contaminating landscape have identified by in situ studies with genotoxins [6]. Determination of DNA damage at the level of chromosome a requisite part of genetic toxicology because of the function of chromosomal mutation in cancer formation [7].

For this reason, biomarkers have used extensively in research programs in recent years and protocols have been established as routine tests [8,9]. Many biomarkers used to detect DNA damage [8,9]. These methods include some genotoxicity tests (a) Structural and numerical chromosomal deviations, sister chromatid changes and micronucleus test, (b) Comet experiments, genotoxicity tests, such as analyzes of DNA adduct. The aim of the genotoxicity tests used in ecotoxicological studies is: Chemical material, (a) To identify the harmful effects on biological systems, (b) Dose-response relationship, (c) Determine the conditions that the toxicant causes. In addition, the toxic effect from the water: (a) Nature View, (b) Qualifications of the, (c) to define the quality.

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Several toxicity tests performed to identify these conditions and the nature of the effect produced.

Today, one of the most reliable genotoxicity tests, the ''Micronucleus Test (MN)'', is frequently preferred in order to quantitatively determination of the biological effect of chemical mutagens on the cellular scale. The micronucleus test is widely used to estimate cytogenetic damage induced by chemical or physical agents. Although most of the works published until now performed this assay on mammalian species (especially rodents), the micronucleus test showed to be a useful tool also with samples taken from non-mammals. In particular, it allows detecting the genotoxic properties of compounds present in the aquatic environment. Both laboratory research (to evaluate the genotoxicity of xenobiotic) and in situ studies (to assess the water quality) have involved several invertebrate species, amphibians and teleosts, such as Cypriniformes, Perciformes, Characiformes, Anguilliformes, Gadiformes, Pleuronectiformes, Salmoniformes and Siluriformes [10-19].

This test has developed in recent years with many aquatic organisms [1,8,9]. It makes possible in determining the remaining chromosomes and broken chromosomes. Due to its advantages, such as, easy to learn, doesn't need to count the chromosomes to observe the chromatids and chromosomal damage hard to detect and see in the metaphase stage, presenting more objective results than other tests in detecting chromosomal damage, possible to count thousands of cells, preparation is fast [10]. The MN test first started to use as a test to determine chemical carcinogens in human cells in the 1970s and then, it employed too many different organisms in order to determine cytogenetic diminution [20-23]. Micronuclei caused by DNA fragmentation in the Interphase cells due to the exposure to contamination. Micronucleus (MN), mitosis are observed in cells that have not been transported to the poles during cleavage, remain, break and/or are composed of all chromosomes and have completed nuclear division [9]. Micronuclei appear when a whole chromosome or a chromosome fragment fails to migrate with one of the two daughter nuclei formed during mitosis. The first case (chromosome loss) is due to and a eugenic event related to the spindle apparatus, while the second takes place after chromosome breakage. These inclusions may see in any type of cell, both somatic and germinal. Therefore, the micronucleus test carried out in any active tissue. Stimulation of epithelial cell division has obtained by damaging the edge of caudal fins [24,25]. The increase in micronucleus counts is indicative of the numerical and structural chromosomal irregularities produced by various agents in cells. Aneuploidy stimulating agents lead to centromeric cleavage errors and malfunctioning of spindle strands, while clastogenes contribute to MN formation by forming chromosomal breaks [9]. Principles of Counting of Micronucleus by Heddle and Countryman [26]. This, MN diameter is smaller than 1/3 of the main core, Dyeing density is the same as the core, Counting of MNs in cytokinesis-blocked dual-nucleated cells only.

Many aquatic organisms (bivalves, crustaceans, sea worms, etc.) Are directly or indirectly linked to the food chain, the exposure of these organisms to carcinogenic or mutagenic agents has led to an increase in the use of such assays in marine organisms. Aquatic organisms can transfer these agents biologically to metabolites accumulate contaminants present in different concentrations in the environment in their tissues and cells. [1,23,27,28]. In order to detect genotoxic activity in the aquatic environment, many researchers have conducted cytogenetic studies with fish that answer to xenobiotic in nearly the same way the mammalians [29,30]. Marine crustaceans may biologically accumulate a number of chemically diverse chemicals that are mutagenic or carcinogenic for man. Mussels, biological indicators in determining genotoxic pollution are preferred in most ecotoxicological studies as they are filter-feeding, live as sessile and are of economic interest. Mutagenicity tests make it possible to detect such chemicals causing pollution in aquatic ecosystem [23,27]. Erythrocyte micronuclei test in fish is a method used in monitoring aquatic pollutants of mutagenic character by using a number of different species [28]. Kligerman reported that many micronuclei existed in fish subjected to pollution [30]. Micronuclei frequency varies depending on the season, type of pollution and fish species. Fish are the most preferred organisms in MN tests because they are the main biomonitor affected by the changing environment where pollutants discharged. Furthermore, they are usually preferred for testing possible genotoxic characteristics of physical and chemical agents because they expose to very diverse chemical substances either directly via water or indirectly via food chain in the ecosystem and because they response to xenobiotic in similar way with mammalians.

In environmental mutagenesis, micronucleus tests give very practical results in monitoring the clastogenic and genotoxic effects of pollutants. These results are mostly obtained from aquatic organisms such as bivalve *Mytilus galloprovincialis*, *Crassotrea gigas* and *Chamelea galina*, fish rainbow trout *Oncorhynchus mykiss, Oreochromis niloticus,* sea urchin, *Paracentrotus lividus* [1-3,31-47]. Boveri, reported that the relation between chromosomal changes and the origin of tumors using developing echinoderm embryos as a model organism [48]. Enhance in frequencies of MN is an indirect marker of structural and numeric chromosomal irregularities cause in the cells by many agents. In situ micronucleus assays have developed for sea urchin [49-55].

Izquierdo et al. performed the MN test in the biliary cells of *Mytilus edulis*, in Madryn containing domestic waste [4]. No effect observed on micronucleus assessment in samples taken as they moved away from polluted stations. When all samples from Gijyon and Arjentin compared, the mean frequency of micronucleus changed since 42 ± 1, 38% to 17, 5 ± 2 and 61%. While the MN frequency was 5.75 ± 1.42% in the samples taken from the nearest zone in the polluted zone. Klobucar et al. conducted the MN test to assess genotoxicity in hemocytes of *Dreissena polymorpha* [55]. For this purpose, samples taken from the Drava River were transferred to the four regions with different concentrations of pollutants in the Sava River (Drava, Zagreb, Oborovo, Sisak, Lukavec) and exposed for 1 month. The lowest level of MN frequency in the mussels hemolymphs was observed in the Drava River, which is the reference region (0.05%), in Lukavec (2.7%), which is contaminated with chemical

wastes in the Sisak region, in Sisak (5.2%), (3.1%) in Oborovo, which is closer to the medium-dirty zone and is most affected by the same contaminants as Zagreb.

In a study by Venier and Zampieron to determine genetic damage in two strains of *Mytilus galloprovincialis* and *Zosterisessor ophiocephalus* found in the Venice lagoon Italy, genetic damage was reported to be present in MN and nucleus abnormalities in hemolymph and gill tissues [56]. In the reported study, MN frequency reported to vary between 33% and 371%. Because of the research, it has revealed that species naturally found in the lagoon exposed to pollution caused by genetic damage.

In a study conducted by Dolcetti and Venier, Mediterranean species *M. galloprovincialis* were investigated for the purpose of determining genetic damage of MN frequencies in living beings exposed to benzopyran both in the natural environment and in laboratory conditions [11]. It is reported that the frequency of micronucleus detected in the gills (about 8.5%) is less than that in the hemolymph (about 48%), when MN formation was detected in lice from different periods and in different periods in the study. And it has been stated that MN frequency increases in parallel with the increase of pollution depending on years.

Dailianis et al. evaluated MN in the hemolymph and gills of *Mytlius galloprovincialis* collected from Thermaikos and Strymonikos (Southern Greece) Gulfs in June and October 2001 [40]. When the samples of June and October were compared, it was observed that there was no significant difference between the seasons as a result of the MN evaluation of the gill texture. In the MN test applied in the hemolymph, it observed that there was no significant seasonal difference between the samples made in June, as in the samples in October.

The MN frequency test has generally applied to organisms where other biological effects, techniques and contaminant levels well documented. In conclusion, the current report indicates that MN test in the aquatic organisms considers sensitive results in monitoring pollution and chemicals and thus, it used as a standard method in regularly monitoring the pollution [57].

Conclusion

Aquatic ecosystems should preserve against all kinds of contrary activities, which may lead to noticeable changes. Genotoxic/carcinogenic compounds accumulated by aquatic organisms might cause health risk for human through by food chain because of their ecological risk of genetic mutation and reduction of genetic diversity. Nevertheless, there has been a little knowledge about the impact of genotoxins on natural human populations. In general, ecologically and economically important aquatic organism could assist as indicator species for Biomonitoring of environmental genotoxicity levels, for screening of genotoxins distribution, or for assessments of genotoxicity effects from contaminant spills or effluent discharge waters.

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