ARTICLE / INVESTIGACIÓN

Studying the toxicity of polluted water with polyaromatic hydrocarbon compound (Anthracene) by using micronucleus assay in fish

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DOI. 10.21931/RB/2023.08.01.51

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Abstract: Polycyclic aromatic hydrocarbons mainly originate from the incomplete combustion of fossil fuels such as petroleum, natural gas and coal. Also, biomass burning has attracted much attention due to its mutagenic, allergenic and carcinogenic properties. Anthracene, a three-ringed polycyclic aromatic hydrocarbon, is widely known as a common hazardous ubiquitous environmental pollutant. Anthracene is used to make dyes, plastics and pesticides. The present study aims to evaluate the risks of Anthracene to fish using a micronucleus (MN) assay; the test has been used successfully as a mutagenic assay. Ninety fishes were adapted and acclimated to the laboratory conditions for one week before starting the experiment, then were exposed to (7.5mg/L, 10mg/L, and 12.5mg/L) of Anthracene for 72 hours. Results demonstrated that the LD50 of Anthracene in fish was (10 mg/L). Based on the values of LC50, the fish were then exposed for 72 h to three concentrations of sub-lethal Anthracene (2.5 mg/L, 5 mg/L and 7.5 mg/L) and control (0.00 mg/L) after (72 hours, 10 days, 20 days). Peripheral blood samples smears were collected from each group, the sample was stained by Giemsa stain, and frequencies of MNs were counted. The study showed an increase in micronuclei with concentration and period. In conclusion, it can use of the micronucleus assay in erythrocytes of fish as a sensible index for the assessment and evaluation of aquatic environmental pollution.

Key words: PAH, Anthracene, Micro nucleus assay, Carp.

Introduction

Polycyclic aromatic hydrocarbons (PAH) are a group of hydrophobic organic compounds that are widespread pollutants. Crude oil drops out, industrial, refinery activities, and civil wastes are essential sources of (PAH) in aquatic ecosystems.PAH are potentially carcinogenic agent[1] and a significant interest because of widespread contaminants in coastal and freshwater zones[2]. PAH contamination is significant near industrialized areas[3].

Anthracene ($C_6H_4.C_2H_2.C_6H_4$) is a rigid polycyclic aromatic hydrocarbon with a low molecular weight derived from coal tar consisting of three fused benzene rings. Anthracene, Para naphthalene, is a Green Oil that accompanies naphthalene in the final steps of coal tar percolation. Anthracene is used in the artificial production of the red dye alizarin. It is also used in insecticides, coating materials and preservatives of wood. Moreover, Anthracene is colorless but exhibits blue fluorescence under ultraviolet light. If Anthracene is released into water, it will be expected to absorb particulate and sediments strongly. It was not hydrolyzed but may be bio-concentrated in aquatic organisms, which causes loss or reduction of microsomal oxidase (this enzyme enables the rapid metabolism of polyaromatic hydrocarbons). It undergoes immediate photolysis close to the surface of natural waters and maybe follows significant biodegradation based on laboratory experiments. Besides, industrial and agricultural activities have raised pollution in the aquatic environment, which is contaminated by different chemicals toxic from the discharge of waste waters and agriculture; these are accountable for several effects on the organisms, including humans[4]. PAHs are absorbed by the fish body surface and via the gills. It also enters through food intake or contaminated materials[5].

Fish are an excellent topic for studying the mutagenic and/or carcinogenic possibility of contaminants in water samples since they can metabolize, concentrate and store waterborne pollutants[6]. Since fish often react to toxicants in a similar way to higher vertebrates, they can be used to screen for chemicals that are potentially teratogenic and carcinogenic in humans. The main application of fish used as a test model is to mark the distribution and effects of chemical contaminants in the watery environment[7]. Micronucleus assay was viable to freshwater and marine fishes and showed that gill cells are more sensitive than hematopoietic cells to micronucleus-inducing agents. The micronucleus assay is an in vivo and in vitro short-time screening procedure that is openly used to discover genotoxic effects. It is one of the most straightforward, dependable, low expenses and fast screening systems[8]. The MN test, one of the most common tests of environmental genotoxicity, has served as an indicator of cytogenetic hurt[9]. Micronuclei are cytoplasmic chromatin masses with the appearance of small nuclei that emerge from chromosome fragments or intact total chromosomes lagging in the anaphase phase of cell division. Their existence in cells reflects structural and numerical chromosomal aberrations arising through mitosis[10]. These compounds may create hepatic lesions and physiological and biochemical disorders in fish. Contaminated fish may then be used to bio-observe the existence and impor-

Citation: Henao-Ramírez, AM.; Palacio- Hajduk, DH.; Urrea-Trujillo, AI. Cost Analysis of Cacao (Theobroma cacao L.) Plant Propagation through the Somatic Embryogenesis Method. Revis Bionatura 2022;7(2) 2. http://dx.doi.org/10.21931/RB/2022.07.02.2
Received: 14 July 2021 / **Accepted:** 10 December 2021 / **Published:** 15 May 2022

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tance of these pollutants. The present study aims to evaluate the risks of Anthracene to fish by using a micronucleus (MN) assay,

Materials and methods

There are 90 fish of common carp (Cyprinus carpio) ranging between 7-9 cm in total length and 60-80 gm. in body weight, with no visible signs of disease or morbidity. Fish were kept in an aquarium with aerated tap water; they were fed on commercial feed and adapted to laboratory conditions for one week before the experiments were complete.

The experiment was conducted in a 20 L aquarium and gently aerated tap water. A set of 10 fish specimens were randomly exposed to anthracene concentrations (7.5, 10.0 and 12.5mg/L); after 72 h, the value of LC50 of Anthracene for C. carpio was specified as 10.0 mg/L.

Based on the LD50 values, the fish were then exposed for 72 h to three concentrations of sub-lethal Anthracene (2.5mg/L, 5mg/L and 7.5 mg/L) and control (0.00 mg/L). The mortalities did not occur in all groups following the period of exposure. After 72 h (Acute exposure), the water was exchanged, and the blood was collected from the caudal vein for a micronucleus test after 72 hrs, 10 and 20 days of the experiment. Statistical analysis was performed by using Excel word 2019

Micronucleus Test

The smear of peripheral blood pulled from the caudal vein by a syringe coated with heparin was prepared, and well-dried slides were stained with 10% Giemsa stain solution for a half hour following the method of (11). (1000 cells/ slide) for micro-nuclei were scored.

Results

The results of this study showed that the MN was increased, correspondingly with the increasing anthracene concentrations (2.5%, 5%, and 7.5%) than that control group in all the anthracene concentrations (Figure 1).

Micronucleus was calculated in the acute group (after 72 hrs), and the chronic group (after 10 and 20 days of the experimental period) showed an increase of micronuclei with the increase in concentration, and also when compared within the group according to the period of exposure as in Figure (2,3,4,5).

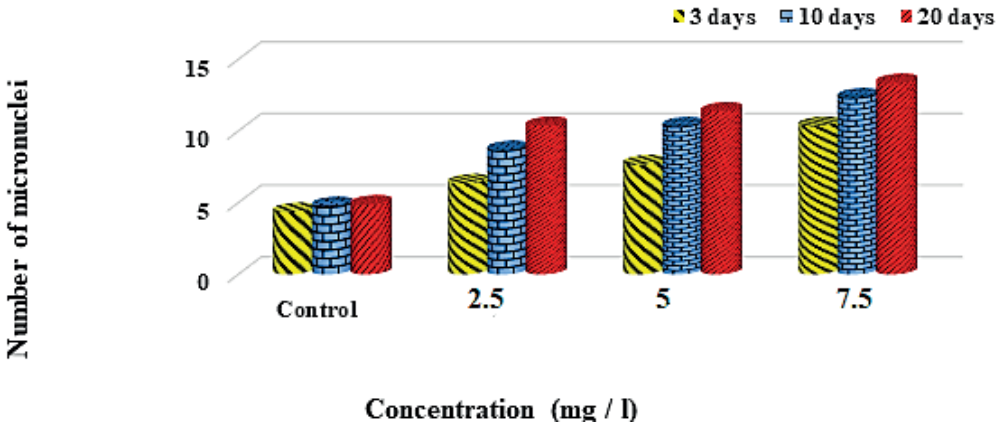

Figure 1. The number of micronuclei at different times and concentrations of Anthracene (mg/L).

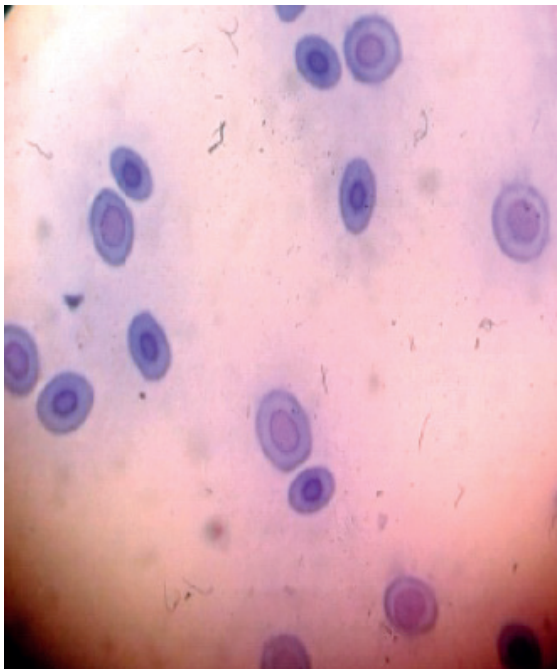

Figure 2. Normal red blood cells without micronuclei.

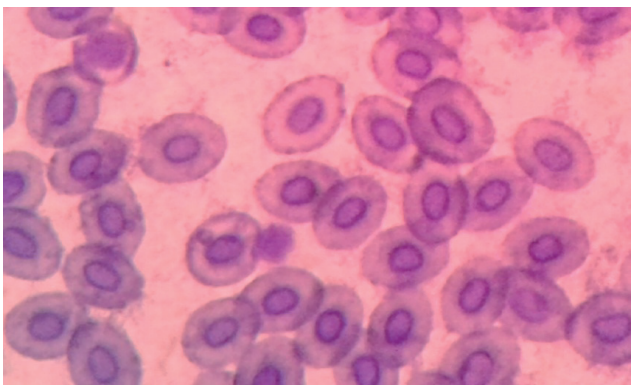

Figure 3. Micronuclei in Red Blood Cells of Fish

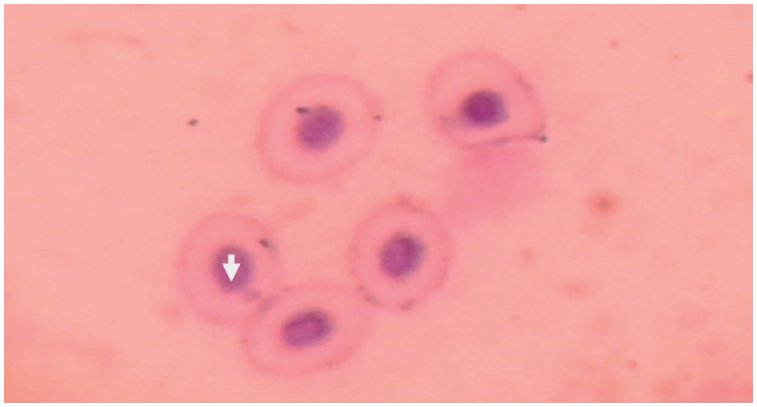

Figure 4. Micronuclei in Red Blood Cells of Fish.

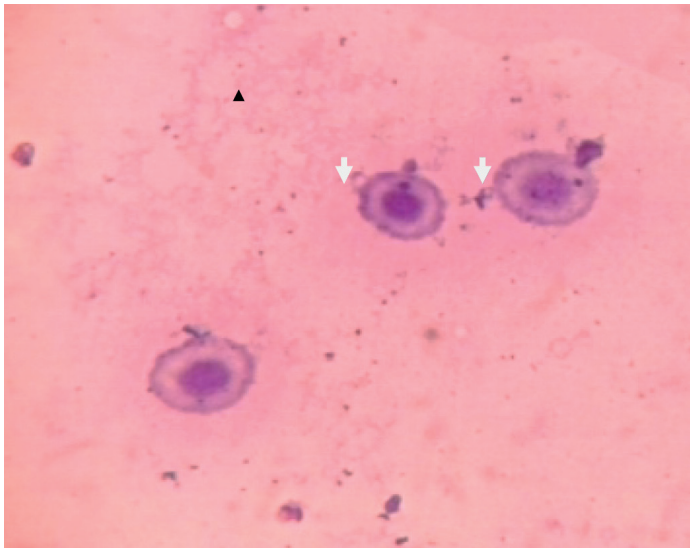

Figure 5. Micronuclei in Red Blood Cells of Fish.

Discussion

The results agreed with the development of Carlos *et al.*[12], who observed an increase in clastogenic accidents, and submission of the formation of MN when considering nuclear alterations and micronuclei together; group xenobiotic exposure and controls were significantly different.

Micronuclei occur due to exposure to different concentrations of benzene and Anthracene. The effect of these structures on the chromosome to form chromosome fragments or loss of centromere lead to delay of these fragments in the anaphase stage cell mitosis. After the telophase stage, these chromosome fragments are turned around to form Micronuclei[13,14].

Differing from other organisms belonging to the trophic chain, fishes are sensitive to relatively small concentrations of environmental Pollutants (with the possibility of mutagenic effects); thus, fishes are considered excellent bioindicators of environmental biomonitoring[15].

MNs assay, initially developed in mammalian species, has been widely used to evaluate the genotoxic effect of many chemical agents[16].

MNs assay can be considered a useful bioindicator of genotoxic and cytotoxic effects of contaminants in aquatic organisms[17-20].

The results showed the importance of MNs assay in mutagenic altered assessment in fishes, which can be used as sentinel organisms to indicate the potential for human exposure to genotoxic chemicals in drinking water. The concentrations of contaminants in fish reverse the situation of contamination of the environment, and thus, the observed levels of total PAHs in fish indicate high levels of PAHs contamination. However, the Consumption rate of 1 g/day appears to be protective from the carcinogenic effects of the PAHs levels. This is because the (Predicted Environmental Concentration) PEC values associated with a consumption rate of 1 g/day are found to be less than the screening value[21].

The present study showed that high PAH levels affect the health of fish and, thus, consumption of these fish may cause a significant health risk.

Conclusions

The present study showed that high PAH levels affect the health of fish and, thus, consumption of these fish may cause a significant health risk. It can use the micronucleus assay in erythrocytes of fish as a sensible index for assessing and evaluating aquatic environmental pollution.

Author Contributions

All authors contributed to the design methodology, analysis results and writing manuscript.

Funding

No funding.

Acknowledgments

We thank the Ministry of Science and Technology / Environment and Water Director / Baghdad /Iraq.

Conflicts of Interest

There is no conflict.

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