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Evaluation of DNA damage in Cyprinus carpio (L. 1758) exposedto malathion using Single Cell Gel Electrophoresis

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ABSTRACT: Malathion (S-(1, 2-dicarboethoxyethyl) O, O-dimethyl phosphorodithioate) is one of the organophosphate pesticides comprehensively used in agriculture fields throughout the world. In spite of widely used, little information are available about DNA damage in aquatic organisms. Therefore, the present study carried out to investigation of DNA damage induced by malathion in Cyprinus carpio (Pisces: Cyprinidae) using single cell gel electrophoresis. The condition of experiment was determined in static system. The specimens were exposed to different non-lethal concentrations (0.5, 1.5 and 3 mgL-1) of the malathion for 96 hour (short-time exposed). Our results showed, the specimens exposed to different concentrations of malathion exhibited significantly higher DNA damage in their blood cells than the control sample ($P<0.05$). This study confirmed that the comet assay is useful method in determining genotoxicity of pesticides. Fish can be used for biomonitoring of the genotoxic pollutants in aquatic environment. Furthermore, DNA strand breakage can be used as biomarker in ecotoxicological studies.

Key words: *Comet assay; Fish; Genetic Damage Index (GDI); Genotoxicity; Insecticides*

INTRODUCTION

High levels of different pesticides used to improve agricultural products and it can be released into aquatic ecosystem in large quantities as runoff from agriculture (Cavas, 2011; Kumar *et al.,* 2010; Nwani *et al.,* 2011). Chemical compounds cause serious hazards on marine and freshwater organisms (Ali *et al.,* 2008). Among different chemicals, organophosphate compounds (such as malathion) which have been highly dissolved in water, biodegrability, slow metabolism in fish and low persistent are mostly employed as pesticides in the world (Ali and Kumar, 2008; Nwani *et al.,* 2010). Most of the organophosphorus pesticides are characterized by their inhibitory action of acetyl cholinesterase (Pandey *et al.,* 2005). Malathion (S-(1, 2-dicarboethoxyethyl) O, O-dimethyl phosphorodithioate) is used in Iran, and it can be detected in aquatic ecosystem (Nasrabadi et al., 2011). The insecticide is more toxic to fish than to mammals due to absence of hydrolytic enzymes in theirs (Sawhney and Johal, 2000). Malathion can affect on fish in different ways as behavioral, reproductive, enzymes and hormonal, developmental, growth, physiological, cellular and genetic effects (Chen *et al.,* 2006), but the information about genotoxic potential of malathion in aquatic organisms is scarce (Kumar *et al.,* 2010).

The comet assay (or Single Cell Gel Electrophoresis) is the best technique to assessment DNA damage due to it's sensitivity (show minimum break, 1 break per 1×1010 Da), rapid, commercial and simple test (Cotelle and Ferard, 1999; Mitchelmore and Chipman, 1998). Among aquatic vertebrate, fish is the best models to assess genotoxic potential of pollutants in aquatic ecosystem (Cavas, 2011; Sumathi *et al.,* 2001), because they have most important position for flow of energy in aquatic food chains and supplied a part of human food that lives in coastal area (Jha, 2004). Furthermore, they bioaccumulate and respond to low concentrations of genotoxic pollutants (Klobučar *et al.,* 2010; Palvica *et al.,* 2011). Peripheral blood cells of fish are great homogenized, therefore usually used to the comet assay (Ateeq *et al.,* 2005; Sponchiado *et al.,* 2010). The present study has been undertaken to describe DNA damage by malathion in *Cyprinus carpio* using comet assay.

MATERIALS AND METHODS

1. Chemical

Malathion (EC 57%) was purchased from local market (manufactured by Partonar Company-Iran).

2. Experimental fish specimen

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The healthy fishes *Cyprinus carpio*, (belonging to the Family: Cyprinidae) were purchased from local fish culture in the north of Iran. The fish average of weigh and length were 15.95 ± 2.41gr and 11.12 ± 0.61 cm, respectively. The specimens were acclimatized under laboratory in semi-static conditions for10 days. Fishes were fed pellet and faecal matter were siphoned off daily to reduce ammonia content in water. 24 hour prior to start of genotoxicity test, feeding was stopped and fishes were not fed during the experimental period.

3. Experimental design

For this study, three non-lethal concentrations 0.5, 1.5 and 3 mgL^{-1} of Malation were selected. 10 aquaria (50 L) were prepared that each aquarium was contained 10 specimens. Sampling was done at 96 hour (4 days) after exposure to malathion at the rate of three fishes per each concentration. Blood samples directly were collected from heart with heparinized syringes (2.5 ml) from each fish and immediately used for comet assay. The study was done based on static method. The comet assay was conducted with whole blood under dim light to prevent UV induced DNA damage.

4. *Single cell gel electrophoresis (SCGE)*

The SCGE or comet assay was performed according to the Tice et al., (2000) with some modification. Blood samples were collected from heart of fish. About 15 µL of the blood was mixed with 145 µL of low-melting point agarose (LMP 0.5%). A 40 µL of the mixture was layered on the microscopic slide which was precoated with normal melting agarose (NMA 1%). The slides kept in refrigerator for 10 min. After solidification, the slides were immersed in cold lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris-base, pH 10, with 10% DMSO and 1% Triton X-100 added fresh) inside refrigerator at 4 ℃ for 1 hour. The slides were placed side by side in electrophoresis chamber containing fresh and cold buffer (300 mM NaOH, 1 mM EDTA, pH 13) for 45 min in refrigerator (4ºC). The electrophoresis was done at 25 V and 300 mA (25-30 min at 4ºC). Observation slides were done with the CETI fluorescent microscope (Model: 3100.5000- Triton II) that equipped with Sony camera (Model: No.DSC-H9). Two slides per specimen were prepared and 50 cells per slide (300 cells per concentration) were scored randomly.

The DNA damage was measured by visual classification of cells into five type "comets" according to the tail length (Cavas, 2011; Lee and steinert, 2003); 0: undamage, 1: low damage, 2: moderate damage, 3: high damage and 4: complete damage. A genetic damage index (GDI), arbitrary units, was employed as below (Grisolia *et al.,* 2009; Silva *et al.,* 2000).

$GDI = (n_1+2n_2+3n_3+4n_4) / (\sum / 100)$

GDI: Genetic Damage Index, n_1: Minimum damage, n_4: Maximum damage, $\sum$: Total number of the cells

5. Data analysis

One-way analysis of variance (ANOVA) was applied to compare mean (±SD) differences in GDI between control sample and treatments. The *p* values less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

The parameters were measured during the experiment included dissolved oxygen, pH and temperature. The average (± SD) was 6.42 ± 0.26 mg/l, 8.34 ± 0/16 and 22.40 ± 0.70 ˚C, respectively. During the experiment no fish mortality was observed. The amount of DNA damage in the cells was estimated from visual classification as extent of material genetically migration and shown by Genetic Damage Index (GDI). The fish specimens exposed to different concentrations of malathion exhibited significantly higher DNA damage in their blood cells than the control group (Table 1; Fig. 1; Fig. 2). Further, the DNA damage was found to be concentration dependent ($P<0.05$). The fish exposed to the lower concentration of malathion (0.5 mgL^{-1}) did not show a significant in the GDI compared to control.

Table 1: Mean (± SD) GDI in blood cells of *C. carpio*

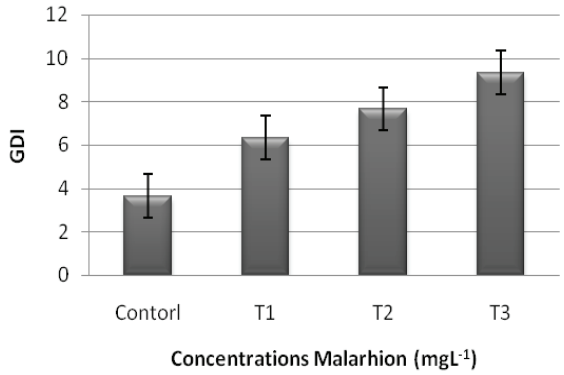

Fig. 1: DNA damage (GDI) in peripheral blood cells of *C. carpio* after exposure to malathion

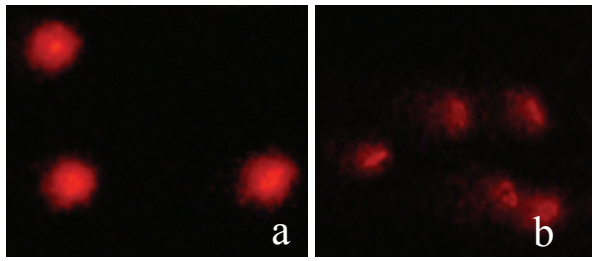

Fig. 2: (a) Control, (b) after exposure to Malathion

The present study showed that the Comet assay can be applied successfully in fish, for assessment of genotoxic potential of malathion. The comet assay under alkaline condition is able to identify DNA damage (e.g. single strand breakage, alkaline labile sites, DNA cross links, etc.) that induced by pesticides (Kumar *et al.,* 2010; Tice *et al.,* 2000). This method have considerable

advantages over the ordinary cytogenetic methods such as chromosomal aberration, sister chromatid exchange and micronucleus test used to detect DNA damage, because in this method the cells do not require to miotically division and chromosomal properties (Ali and Kumar, 2008; Nwani *et al.,* 2010; Pandrangi *et al.,* 1995). Moreover, the assay is useful method for *in vivo* and *in vitro* Genotoxicology on aquatic organisms (Cotelle and Ferard, 1999). According to several study, the comet assay has been successfully applied in blood cells of many fish species. So, Fish are suitable tools for monitoring of genotoxic pollutants in aquatic environment (Abdul-Farah *et al.,* 2003; Cavas and Ergene-Gözükara, 2005). Our results showed that the DNA damage for concentrations of malathion tested where significantly higher than control and indicated the genotoxic potential of malathion on *Cyprinus carpio*. The results are in agreement with the finding of Kumar *et al.,* (2010) that observed malathion can be caused DNA damage on *Channa punctatus*. Kushwaha *et al.,* (2000) showed DNA damage in *C. punctatus* following exposure to malathion. Also, The results are in accordance with other researchers who worked on human. Moore *et al.,* (2010) reported malathion induced cytotoxic and genotoxic effects to human liver carcimona cells. Blasiak *et al.,* (1999) observed DNA damage in human lymphocytes exposed to malathion and its two analogues. The DNA damage detected in this study could have originated from DNA single-strand breaks, DNA double-strand breaks, DNA adducts formation and DNA-DNA and DNA-protein cross links (Mitchelmore and Chipman, 1998), resulting from the interaction of pesticide or their metabolites with genetic material (Garaj-Vrhovac and Zeljezic, 2000). The DNA damage, especially DNA single-strand breaks, can be used as biomarker for identified of genotoxic pollutant in aquatic ecosystem. In general, organophosphate pesticides when undergo different metabolic processes inside a living organisms (such as fish) produces a lot of free radicals that very often can interfere with the bimolecules structural like protein and nucleic acids (Mohanty *et al.,* 2011). The mechanism of DNA damage due to malathion exposure is poorly understood and little is known about malathion or its metabolites which are responsible for induce DNA damage. Malathion or its metabolites are two electrophilic groups: the alkyl groups and phophoryl groups that are a good substrate for nucleophilic attack. This may interact to the different way with DNA such as phosphorilation process (Ali *et al.,* 2009; Blasiak *et al.,* 1999). In addition, it seems that oxidative stress have an important role to induce cytotoxic and genotoxic damage (Moore *et al.,* 2010).

CONCLUSION

Comet assay is sensitive for the detection of DNA damage in aquatic organisms. Therefore, the assay can be applied for investigation of genotoxic pollutants. Our results demonstrated that malathion can be induced DNA damage in fish. Based on the results, we may suggest careful use of malathion to protect against genetic damage to aquatic organisms and also to human population. Several studies demonstrated genotoxicity of malathion both *in vivo* and *in vitro*, but the reports are conflicting. More studies are needed to investigation DNA damage in aquatic organisms after exposure to malathion.

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