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Original Article

DNA damage and hematological changes in Common carp (*Cyprinus carpio*) exposed to oxadiazon

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Abstract: This study was carried out to investigate the genotoxic, and hematological and serum biochemical effects of a widely used herbicide, oxadiazon in common carp (*Cyprinus carpio*) fingerling. Fish were exposed to different concentrations (0, 1, 1.5 and 2 ppm) of the herbicide for 30 days. Blood samples were collected, then comet assay in circulating erythrocyte cells was applied. Erythrocytes cells of fish exposed to 1, 1.5 and 2 ppm of oxadiazon showed DNA damage (21.3%, 22.9%, and 28.4%, respectively) significantly higher than the control group. Moreover, exposure to oxadiazon significantly decreased WBC, RBC, Hb, Hct as well as serum albumin, glucose, and total protein levels, while serum ALP was significantly increased in the exposed fish groups. No significant differences were found in MCV, MCHC and MCH levels between oxadiazon treatments and control groups. In conclusion, this study shows that oxadiazon is highly toxic to *C. carpio* and causes significant changes in hematological and biochemical parameters as well as indicates the mutagenic potential of oxadiazon in the erythrocyte cells of this fish.

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Introduction

Herbicides can be introduced into aquatic environment due to runoff and leaching, cussing adverse effects on non-target organisms, particularly fishes (Wany et al., 1992). Oxadiazon (Ronstar) is a widely used herbicide in rice fields against both mono and dicotyledonous weeds, as well as in fruit trees, vines, grasses, cotton, soybeans, onions, and sunflowers (Ahmed et al., 2008). Different oxadiazon residue concentrations ranged 0.4 to 7.24 µg/l in different water bodies (Mamun et al., 2009; Kim et al., 2014), as well as up to 0.442 ppm in different tissues of fishes and shellfishes have also been reported (Imanaka et al., 1981).

Exposure to oxadiazon have been revealed a retarding growth in African catfish (*Clarias gariepinus* (Ajani et al., 2015), adverse effects on serum biochemical profile in common carp (*Cyprinus carpio*) (Saravanan et al., 2017) and Platy fish (*Xiphophorus maculatus*) (Sadeghi and Imanpoor, 2015), and induced peroxisome proliferation in the rodents (Richert et al., 1996). Exposure of aquatic organisms even to low environmentally-relevant concentrations of pesticides can result in severe effects on genetic and physiological parameters that can be considered as biomarkers for evaluation of fish health as well as monitoring of environment pollutants (Wendelaar-Bonga, 1997; Blahova et al., 2014; Ahmadivand et al., 2016; Mitkovska et al., 2017).

Comet assay is a sensitive technique for detection of DNA damage, practically applied in all nuclear eukaryotic cells, especially for biomonitoring and confirming DNA damage in aquatic organisms (Jin et al., 2004; Kim and Hyum, 2006; Klobucar et al., 2010; Mitkovska et al., 2017). The method allows to detect a wide variety of DNA damage, including DNA single-strand breaks, double-strand breaks, alkali-labile sites and reparation, as well as oxidatively induced base damages, even when exposed to low concentrations of toxicants (Lee and Steinert, 2003; Frenzilli et al., 2009).

Common carp is one of important and valuable

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commercial fish species in Iran that farmed in the Caspian Sea basin of Iran, area that high amount of herbicides are used (Salehi, 1999). Moreover, this species is widely used in the evaluation of physiological and genotoxic effects of pesticides in both laboratory and field conditions (Poleksic and Karan, 1999; Jin et al., 2004; Kim and Hyum, 2006; Klobucar et al., 2010; Blahova et al., 2014; Mitkovska et al., 2017). In this study, we examined the DNA damage in erythrocyte cells of common carp fingerling using the comet assay, and its hematological and serum biochemical changes after 30 days exposure to different concentrations (1, 1.5 and 2 ppm) of the herbicide, oxadiazon.

Materials and Methods

Chemicals: For this study, oxadiazon (12% EC) manufactured by Behkesht Company (Tehran, Iran) was used. The stock solution was prepared in acetone and tap water based on the manufacturer's protocol.

Fish: Common carp with the mean weight of 18.27±2.3g and body length of 11.4±0.7 cm were obtained from a local fish farm (Guilan, Iran), and acclimated to laboratory conditions in 1000 L tanks filled with dechlorinated tap water for two weeks. The fish were fed commercial FFC-Extruded fish food (Faradaneh Company, Iran) twice a day and starved for 24 h before sampling.

Experimental design: A number of 120 fish were selected and divided into four duplicate groups (15 fish per replicate) in 100 L tanks and were exposed to concentrations 0, 1, 1.5 and 2 mg/l of oxadiazon. During the experiment the physicochemical characteristics of the water including, water temperature (°C), dissolved oxygen (mg/l), pH, and total hardness (mg/l as $CaCO_3$) were 25.4±0.9, 6.5±4, 7.8±0.1 and 110±5, respectively. The water was renewed daily and the dead fish were counted and removed.

Hematological and serum biochemical analysis: After 30 days exposure to different concentrations (0, 1, 1.5 and 2 mg/l) of the oxadiazon, five fish from each replication were anesthetized with clove powder (200 mg/l), and blood was collected from caudal vein puncture. Hematological parameters including red blood cells (RBCs), white blood cells (WBCs), hematocrit (Ht) and hemoglobin (Hb), mean erythrocyte volumes (MCV), mean color concentration (MCHC) and mean erythrocyte hemoglobin (MCH) were determined based on Svobodova et al. (1991). Serum alkaline phosphatase (ALP), glucose, total protein and albumin were determined by commercial kits (Parsazmon Co. Iran) according to the manufacturer protocol.

Comet Assay: The DNA damage in the collected blood samples of exposed carp was determined by comet assay (alkaline single cell gel electrophoresis) method as previously described by Singh et al. (1988). Briefly, a mixture of 5 μL of blood sample with 95 μL of 0.5% (w/v) low-melting agarose was added into degreased microscope slides previously covered with 1% normal melting agarose and covered with a coverslip. After agarose solidification (4°C for 20 min), the embedded cells were lysed in lysing buffer (2.5M NaCl, 100mM Na_2EDTA, 10mM Tris-HCl, 1% Triton X-100 and 10% DMSO, pH=10) at 4°C overnight. After a 30 min incubation in electrophoresis buffer (300 mM NaOH, 1 mM EDTA, pH≥13) electrophoresis was carried out at 20 V and 300 mA for 30 min. Subsequently, neutralization was performed in three washing steps in 0.4 M Tris-HCl (pH=7.5). To visualize DNA strand breaks, slides were stained with ethidium-bromide and observed using a fluorescence microscope (E600; Nikon). The DNA damage was quantified as the percent of tail DNA.

Statistical analysis: The data was analyzed statistically at $P<0.05$ by one-way analysis of variance (ANOVA) using the SSPS 20 software (Chicago, IL, USA).

Results

Hematological and serum biochemical parameters: The results of hematological and serum biochemical parameters are shown in Table 1. The WBC levels of fish exposed to 1.5 and 2 ppm were significantly lower than the control group ($P<0.05$), however, no significant changes were observed in the fish exposed to 0.1 mg/l of oxadiazon. Similarly, significant

Table 1. Hematological and serum biochemical parameters of *Cyprinus carpio* after 30 days exposure to different concentrations (0, 1, 1.5 and 2 ppm) of oxadiazon.

Dose (ppm)	0	1	1.5	2
WBC (10^3/µL)	33±1.99[a]	31.03±0.4[a]	28.26±0.49[b]	27.16±0.56[b]
RBC (10^6/µL)	1.24±0.2[a]	1.20±0.4[a]	1.17±0.21[a]	1.03±0.5[b]
Hb (g/dl)	6.9±0.36[a]	6.56±0.24[a]	5.7±0.26[b]	4.07±0.58[c]
Ht (%)	33±1[a]	31.1±0.68[ab]	30.2±1[b]	25.83±1.4[c]
MCV (fl)	227.39±1.61[a]	224.44±6.4[a]	228.76±2.28[a]	231.76±16.73[a]
MCH (Pg)	66.22±1.53[a]	65.69±0.98[a]	65.08±1.51[a]	64.63±4.43[a]
MCHC (%)	20.93±1.57[a]	21.11±0.47[a]	18.90±1.49[a]	18.73±1.92[a]
ALP (U L)	64.33±2.51[b]	75±4.35[a]	70.66±6.65[a]	80.33±7.09[a]
Albumin (g/ dL)	0.96±0.02[a]	0.63±0.02[d]	0.78±0.02[b]	0.72±0.01[c]
TP (g/ dL)	2.74±0.02[a]	1.97±0.05[d]	1.83±0.02[b]	1.45±0.03[c]
Glucose (mg/ dL)	89.33±7.02[a]	58±1[b]	59.33±1.15[b]	55.33±4.73[b]

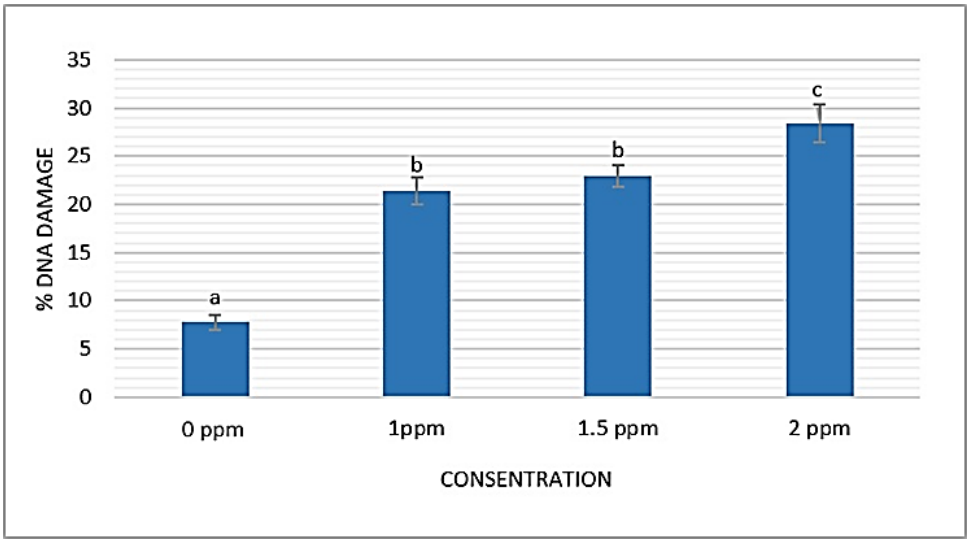

Figure 1. DNA damage in the blood samples of common carp on day 30 of exposure to different concentrations (0, 1, 1.5 and ppm) of oxadiazon. Different letters indicate significant differences between the groups at $P<0.05$.

decreases in hematocrit and hemoglobin levels were only found in fish exposed to 1.5 and 2 ppm. Also, exposure to the highest concentration (2 ppm) of oxadiazon significantly decreased RBC levels ($P<0.05$). No significant differences were found in MCV, MCHC and MCH levels between oxadiazon treatments and control groups ($P>0.05$). Moreover, exposure to oxadiazon significantly decreased serum albumin, glucose and total protein levels, while serum ALP was significantly increased in the exposed fish groups ($P<0.05$).

DNA damage: The results of the DNA damage (% tail DNA) in the erythrocyte cells of the control and treated groups are shown in Figures 1 and 2. Cell viability was found to be more than >80% in all treatments, allowing the comet assay to be performed. Almost (92.5%) of the erythrocyte cells in the control group presented no DNA damage, however, that the fish specimens exposed to different test concentrations exhibited significantly higher DNA damage ($P<0.05$). Among the tested concentrations, the highest damage (28.4%) was observed in erythrocyte cells of fish exposed to 2 ppm of oxadiazon followed by 1.5 ppm (22.9%) and 1 ppm (21.3%) trial groups.

Discussion

This study describes genotoxic effects, and hematological and serum biochemical changes in common carp exposed to different concentrations of the herbicide oxadiazon. Our findings confirmed the potential of oxadiazon herbicide to induce DNA damage in erythrocytes cells of this fish. For assessment of genotoxic contamination of the aquatic environment, Klobucar et al. (2010) and Mitkovska et

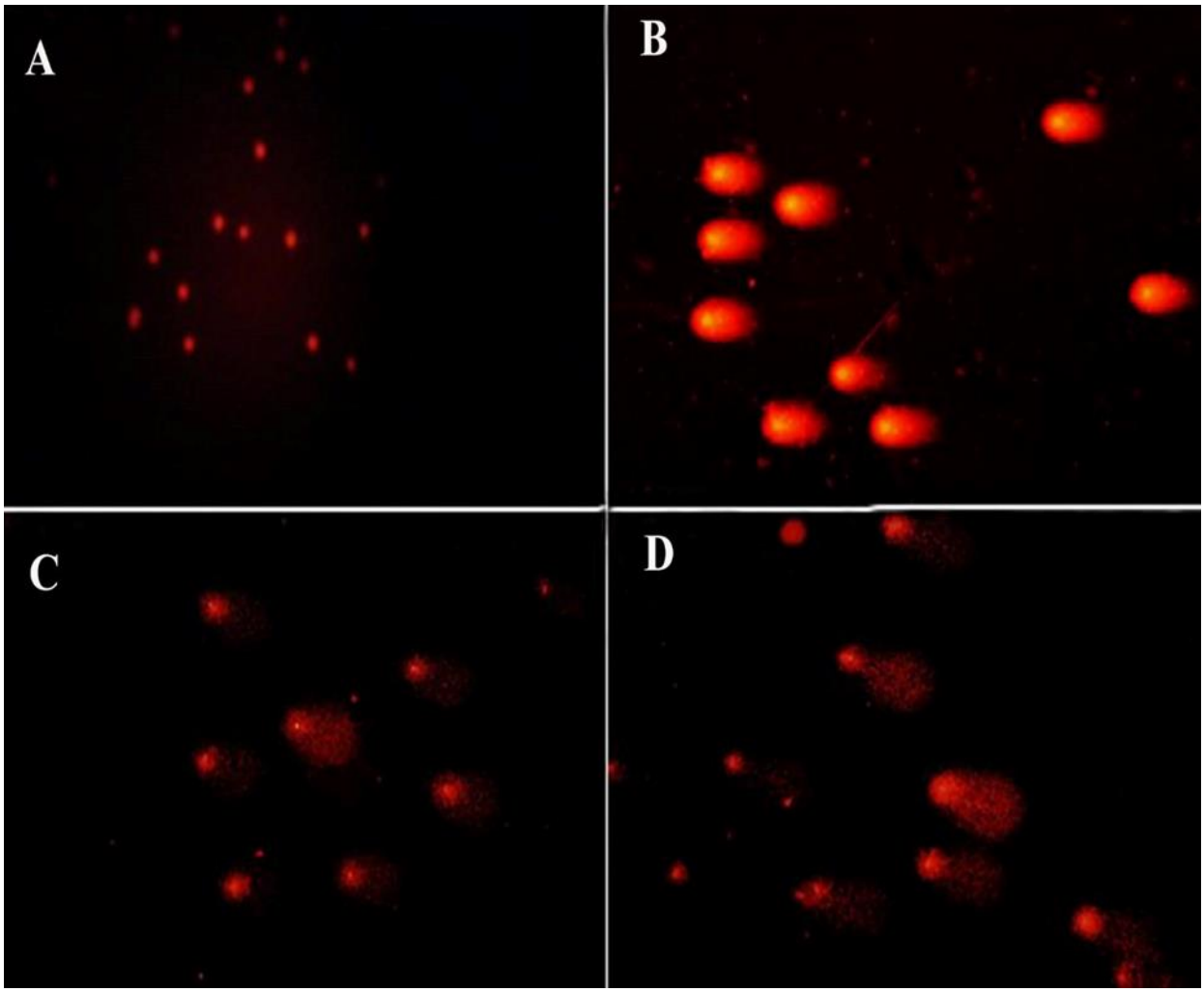

Figure 2. Comet assay of blood samples from common carp showing grades of DNA damage in erythrocytes after 30 days exposure to oxadiazon. A: Control; B: 1 ppm; C: 1.5 ppm, and D: 2 ppm.

al. (2017) found *in vivo* genotoxicity by comet assay in carp, confirming this method as a reliable biomarker and common carp as a suitable bioindicator for water quality. Many studies, assessing the genotoxic effects of pesticides found significant increase in the DNA damage of erythrocytes by enhancing concentrations and exposure time (Cavas and Konen, 2007; Guilherme et al., 2012; Moreno et al., 2014). In contrast, Cavalcante et al. (2008) observed that DNA damage in gill cells of *Prochilodus lineatus* exposed to Roundup was not persisted over time.

In the current study, the incidence of DNA damage in erythrocytes cells after 30 days of exposure to oxadiazon could be attributed to intrinsic differences in the repair enzyme system and/or turnover cell in erythrocytes. However, further studies are still necessary to confirm this hypothesis.

In response to a stressor such as pesticide exposure, the fish undergo a series of biochemical and hematological changes in an attempt to compensate the challenge imposed on them and thus cope with stress (Wendelaar-Bonga, 1997). Similarly, significant changes in hematological and serum biochemical parameters of oxadiazon exposed fish were observed in this study. Saravanan et al. (2017) assessed the acute toxicity effects of 0.5, 5 and 50 µg/l concentrations of oxadiazon on carp for 96 h, found that this herbicide causes a significant decrease in RBC, Hb, and Hct whereas the MCV, MCH, WBC and serum ALP were higher in treated group. However, the value of total protein, albumin, globulin, and MCHC did not show any change in treatments. The effects of oxadiazon on some hematological and

serum biochemical parameters in the recent report (Saravanan et al., 2017), are in contrast with our finding which may be due to exposure time, herbicide concentration as well as fish health condition.

The decrease RBC, Hct and Hb content in this study could be explained as a compensatory response that reduces the oxygen carrying capacity to maintain gas transfers and indicates a change in the water blood barrier for gas exchange in the gill lamellae (Jee et al., 2005). Also, change in WBC levels observed in current study indicates the immunotoxic potential of the herbicide as well as its xenoandrogens activity since oxadiazon has targeted the leukocytes profile (Milla et al., 2011; Ahmadivand et al., 2015).

Moreover, change in serum ALP, albumin, glucose and total protein levels might have resulted from the hepatic dysfunction and immunosuppressive effect of the herbicide (Nayak et al., 2004).

In summary, this study shows that oxadiazon is highly toxic to the fish organism and causes significant changes in hematological and biochemical parameters as well as indicate the mutagenic potential of oxadiazon in the erythrocyte cells of *C. carpio*, suggesting that its use should be carefully monitored considering its potential impact on aquatic fauna. The current study also indicates that the comet assay is very sensitive tools for evaluating the genotoxic effect of pesticides on fish as well as further recommends its combined use with other biomarkers to monitoring aquatic environmental pollutions. Further studies are necessary to elucidate its toxic effects on different biological parameters of fish, especially reproduction and immune system.

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چکیده فارسی

بررسی اثرات سم اگزادیازون بر میزان آسیب DNA و پارامترهای خون‌شناسی در ماهی کپور معمولی (*Cyprinus carpio*)

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چکیده:

این مطالعه با هدف بررسی اثرات علف‌کش اگزادیازیون بر میزان آسیب DNA و همچنین پارامترهای خون‌شناسی و بیوشیمیایی در ماهی کپور معمولی (*Cyprinus carpio*) انجام شد. بدین منظور تعداد ۱۲۰ عدد ماهی کپور (میانگین وزنی ۲/۳± ۱۸/۲۷ گرم و طول ۰/۷± ۱۱/۴ سانتی‌متر) در چهار گروه هر کدام با دو تکرار و تراکم ۱۵ ماهی در تانک های ۱۰۰ لیتری به مدت ۳۰ روز در معرض غلظت های ۰، ۱، ۱/۵ و ۲ میلی‌گرم در لیتر سم اگزادیازیون قرار گرفتند. در انتهای دوره آزمایش از ماهیان نمونه خون تهیه و پارامترهای خون‌شناسی و بیوشیمیایی سرم بین گروه‌ها مقایسه شد. همچنین میزان آسیب DNA در سلول‌های گلبول قرمز با استفاده از آزمون کامت و تعیین میزان درصد DNA دنباله‌دار بررسی گردید. بر اساس نتایج میزان آسیب DNA در تیمارهای سم بطور معنی‌داری بیشتر از گروه کنترل بود ($P<$ ۰/۰۵). بیشترین میزان تخریب در غلظت ۲ میلی‌گرم برلیتر (۲۸/۴ ٪) و سپس به‌ترتیب در غلظت‌های ۱/۵ و ۱میلی‌گرم بر لیتر (بترتیب ۲۲/۹ ٪ و ۲۱/۳٪) مشاهده شد. به‌علاوه در گروه‌های تیمار اگزادیازیون میزان هماتوکریت، هموگلوبین، تعداد گلبول‌های قرمز، تعداد گلبول‌های سفید و همچنین میزان گلوکز، پروتئین کل و آلبومین سرم کاهش معنی‌داری مشاهده شد، در حالی که این گروه‌ها افزایش معنی‌داری در میزان آنزیم کبدی آلکالین فسفاتاز نشان دادند ($P<$ ۰/۰۵). تغییر معنی‌داری در میزان MCH، MCHC و MCV در بین گروه‌های آزمایش مشاهده نگردید ($P>$۰/۰۵). بر اساس نتایج این مطالعه اگزادیازیون برای ماهی کپور بسیار سمی و دارای قابلیت ژنوتوکسیکی بوده و اثرات شدیدی بر پارامترهای خون‌شناسی و بیوشیمیایی این ماهی می‌گذارد.

کلمات کلیدی: اگزادیازیون، کپور، آزمون کامت، فاکتورهای بیوشیمیایی.