

Toxic effects of dechlorane plus on the common carp (*Cyprinus carpio*) embryonic development

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Research

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Abstract

Dechlorane Plus (DP) is a widely using chlorinated flame retardant, and has been detected extensively in the environment. Even though the DP content in the surface water is low, it can pose a continuous exposure risk to aquatic organisms due to its strong bioaccumulation. Since related researches on the toxicity mechanism of DP exposure on carp embryo development are limited, we carried out the evaluation of DP effect on carp. In this study, Carp embryos were exposed to DP of 0, 30, 60, and 120 µg/L at 3h post fertilization (hpf). The expression of genes associated with the neural and skeletal development such as *sox2*, *sox19a*, *Mef2c*, and *BMP4* were evaluated using quantitative PCR (qPCR), and analysis of different developmental toxicity endpoints changes were observed with microscopy. Our results demonstrated that the expression of *sox2*, *sox19a*, *Mef2c*, and *BMP4* were significantly changed and several developmental defects were observed in the exposed carps embryo, such as DNA damage, increased mortality rate, delayed hatching time, decreased hatching rate, decreased body length, increased morphological deformities. In addition, reactive oxygen species (ROS) and malondialdehyde (MDA) were significantly increased with DP exposure at 60µg/L and 120 µg/L. Altogether, our results suggest that DP may have an unique modes of action, which results in aberration occurrence in the early development stage of common carps, that may relates to some gene damage and oxidative stress. Besides, the parameters evaluated here can be used as tools to access the environmental risk for biota and humans exposed to DP.

Background

Dechlorane Plus (DP) is a polychlorinated flame retardant designed to replace Brominated flame retardants or Mirex (Ji et al., 2018), and it is widely used in plastic roofing materials, electrical wires, hard disk connectors of computers, and other polymeric systems (Ren et al. 2008). DP is found common in the aquatic environment (Li et al. 2014; Sun et al. 2017; Tao et al. 2015; Wu et al. 2016), with a half-life of about 24 years in the water environment. It is a typical persistent organic pollutant and has an effect of bio-enrichment and bio-fortification in the biota (OxyChem 2007; Yu et al. 2013). Hoh et al. first discovered the existence of DP in the environment in 2006. Some studies show that DP accumulates in female herring gulls, crucian carp, ducks, lake trout, and mud carps, and that it is maternally transferred to their eggs (Guo et al. 2017; Gauthier and Letcher 2009; Wu et al. 2016). Studies have also shown that DP possesses physiological toxicity. For example, after 90 days of DP exposure, the mRNA expression level of CYP2B2 in rats decreased, and the enzyme activity is increased (Li et al. 2013a). In addition, orally feed DP can cause metabolic disorders, signal transduction alteration, and liver oxidative damage in rats (Wu et al. 2013). DP can also induce spontaneous movement of zebrafish embryos, with significantly reduced free style speed and significantly reduced larval swimming speed (Chen et al. 2017). DP can also induce DNA damage in *Tetrahymena thermophila* and marine bivalves ((Dou et al. 2015; Baron et al. 2016).

Sox genes play important roles in growth, development, and physiological processes. The main function of these genes is to participate in neurogenesis, bone formation, stem cell development in the embryo, cardiogenesis, sex determination, and hematopoiesis in zebrafish (Akiyama et al., 2004; Avilion et al.,

2003;Pevny and Placzek, 2005; Polanco and Koopman, 2007; Schilham et al., 1997; Smit et al., 2001). Sox2 gene encodes a transcription factor which plays an important role in maintaining pluripotent stem cell populations, early development, and differentiation in zebrafish (Okuda et al., 2010). The sox19 gene also plays a very important role in embryogenesis. Studies show that the sox2, sox3, and sox19a genes knocked down results in abnormalities in the central nervous system and in the tail region of zebrafish embryos (Okuda et al., 2010). Mef2c gene is particularly important in early development of zebrafish heart (Hinitz et al. 2012) and of mice skeletal muscle (Potthoff et al. 2007). Verzi et al. (2007) found that lacking Mef2c in neural crest lineage causes jaw defects in mice. BMP4 gene is involved in the process of chondrocyte differentiation into cartilage and gives rise to bone in the differentiation and maturation of cells of osteoblastic lineages (Balemans and Van Hul 2002; Canalis et al. 2003; Hogan 1996; Graff 1997; Mariani and Martin 2003; Thies et al. 1992; Wan and Cao 2005; Yoon and Lyons 2004). BMP4 signaling inhibition leads to sclerotic maturation or delayed function of bones (Xia et al. 2018). However, little is known about the sensitivity of early developmental stages of fish to DP.

Common carp, *Cyprinus carpio* L. (*C. carpio*) is commonly used in ecotoxicology studies. It is a very important organism in the point of ecological and economic. The *C. carpio*, is the most widely farmed freshwater fish in China. It is reported that *C. carpio* is often used as a biological indicator species to understand the impact of environmental pollution (Burcu & Cahit, 2014). The objectives of the present work were to characterize the main organic contaminants present in water environment, to evaluate the toxicity produced by that polluted water on the embryonic development of the *C. carpio*, and to correlate the morphological changes found with the production of gene expression and DNA damage. Thus, we analysis the ecotoxicological threat of DP in the early development stage of common carps, and assess the potential dangers that DP may spread to aquatic organisms.

Materials And Methods

Chemicals

Dechlorane Plus (CAS No. 13560-89-9; purity > 97%) was purchased from Jiangsu Anbang Electrochemical Co., Huai'an, China. Dimethyl sulfoxide (DMSO) (CAS No. D8371; purity > 99.5%) was procured from Beijing Solarbio Science & Technology Co. Ltd., Beijing, China. All solvents used in this experiment were ultra-residue analytical grade (purity > 99%). Dissolving DP in DMSO to prepare DP stock solution, working solutions of 30, 60, and 120 µg/L were freshly prepared by a 1000-fold dilution of the corresponding stock solution with water (Hallare et al., 2006). The final DMSO concentration was 0.1% (v/v) across all exposure groups. Additionally, groups with aerated water and DMSO (0.1% (v/v)) were used as controls. Studies have demonstrated that 0.1% (v / v) DMSO exposure couldn't cause any developmental defects in zebrafish embryos (Hallare et al., 2006).

Collection of fertilized eggs

Fertilized eggs of common carp were obtained from the College of Fishery, Henan Normal University, Xinxiang, Henan Province, China. The embryos were transferred to a petri dish with a diameter of 100 mm for toxicity testing. The experimental use of fish was approved by the Animal Ethics Committee of Henan Normal University (HNSD No. 20180901-0915-01).

Exposure for embryos toxicity

Petri dishes containing 300-350 healthy embryos were exposed to DP at concentrations of 0, 30, 60, and 120 µg/L for 120 hpf. Embryos were maintained in an incubator at 26 ± 1 °C with a photoperiod of 14:10 h light: dark. This study used larval mortality, hatching rate, malformations and body length as toxicological endpoints. Direct observation of embryos in a petri dish under a stereo microscope and the above endpoints were scored at 24, 48, 72, 96 and 120 hpf. All experiments were conducted triplicate, and the exposure solution of the same concentration was replaced every 12 h, while the dead embryos and juveniles were removed in a timely manner. Each treatment group was assayed in triplicate, and all the tests were independently repeated twice to ensure reproducibility. Hatching rate was calculated as the percentage of eggs hatched in a specific time, whereas the mortality rate was calculated as the percentage of dead eggs in all eggs in a given time. Larvae of 120 hpf were positioned, photographed and measured for body length, the deformation rate calculated as percentage of deformation rate of live eggs at 120 hpf. In order to ensure the developmental synchronization in the early stage of DP exposure, the embryos were exposed 3 h post fertilization (hpf) after the fertilization (blastocyst stage).

Measurement of ROS and MDA generation

Determination of ROS and MDA content were conducted by using ROS assay kit and MDA Assay kit respectively, by following the manufacturer's instructions (Shanghai Yiyuan Biological Engineering Co., Shanghai, China). ROS generation in larvae at 96 hpf was detected by using dichlorofluorescein-diacetate (DCFH-DA) with a fluorescence microplate reader (Varioskan Flash, USA) with excitation and emission at 485 and 528 nm, respectively and was shown as DCF/mg protein. MDA, which is produced during fatty acid degradation and is indicative of lipid peroxidation, was quantified based on the method of TBA (thiobarbituric acid) and was shown as nmol/mg protein.

Quantitative PCR (q-PCR)

Total RNA was prepared by using TRIzol reagent (TransGen Biotech, Beijing), then was reverse transcribed to complementary DNA by using a PrimeScript RT reagent Kit (TaKaRa Biotechnology, Dalian, China). Real-time quantitative PCR (qPCR) was done on an ABI 7500HT sequencer (Applied Biosystems, Foster City, USA). The sequences of gene including four embryonic developmental genes (*sox2*, *sox19a*,

Mef2c, and *Bmp4*) were downloaded from the NCBI database. The primers were designed using Primer primer 5 (Table 1). *β-actin* was used as an internal control to normalize the relative mRNA expression of a specific gene via the $2^{-\Delta\Delta CT}$ method (Livak & Schmittgen, 2001).

Comet assay

To detect cellular DNA damage, a comet assay was performed according to protocols developed by Singh et al. (1988) with minor modifications. Mechanical cell isolation, the first step of the comet assay, was carried out as described by Kosmehl et al. (2006). At 24 hpf, embryos with at least 90% cell viability were selected for the comet assay. To visualize DNA damage, 100 nuclei per slide were measured at 40× magnification. The results were assessed based on tail length, tail DNA (%), and olive tail movement (OTM) using the CytoVision NT automatic (Beijing Biolaunching Technologies Co., Ltd., Beijing, China) image analysis system. Percentage tail DNA, considered the most valuable parameter was recorded (Praveen Kumar et al., 2014). All the experimental were represented in three replicates.

Statistical analysis

All assays were carried out in at least in triplicate, data are shown as mean ± standard error of the mean (SEM). All data were verified for normality and homogeneity of variance using KolmogoroveSmirnov one sample test and Levene's test. Comet assay data (% tail DNA) were arcsine transformed and tested for normality and homogeneity using the Shapiro-Wilk test and Levene's test respectively. The correlation between DNA damage and abnormalities in embryo development was analyzed by the Pearson coefficient test. All statistical analyses were conducted using SPSS 18.0. (IBM, USA). The level of statistical significance was set at $p < 0.05$, indicated by an asterisk.

Results

Developmental toxicity

The average mortality rate of DP exposed embryos was recorded in Fig. 1A. It was shown that the mortality rate of embryos increases over time with the same concentration of DP. The mortality rate was significantly decreased ($P < 0.05$) only in the 30 µg/L group at 24 hpf. However, the cumulative mortality of embryos was significantly higher ($P < 0.05$) in the 60, and 120 µg/L treatment groups in contrast to the control group at 48 hpf and 96 hpf. There was a significant increase ($P < 0.05$) in the mortality of embryos in the 120µg/L treatment groups at 72 hpf.

Hatching rate of embryos varies according to exposure concentrations and exposure time (Fig. 1B). With the DP concentration increasing, the hatching rate of the common carp embryos decreased significantly ($P < 0.05$), which is inversely proportional to the DP concentration. However, with exposure time increasing, the hatching rate of embryos in DP treatment group increased. Interestingly, the embryo hatching rate in the 120 $\mu\text{g/L}$ treatment groups was consistent at 72 hpf and 48 hpf, indicating the delay in the hatching process is attribute to DP toxicity.

Frequency of morphological deformities induced by different concentrations of DP exposed to common carp embryos at 120 hpf is shown in Fig. 2A. With the exposure time prolonging, the embryo malformation rate increased significantly ($P < 0.05$), which indicating that the DP initiated embryo developmental toxicity in a time-dependent manner. The typical malformations including pericardial edema (PE), body malformations (BM) (including tail deformation and short tail), spinal curvature (SC), and other malformations were explored (Fig. 2B).

The size of the exposed and control groups of larvae were measured at 120hpf and are shown in Supplementary data: Fig S1. Exposed larvae exhibited a concentration dependent decrease of total body length. Control groups of larvae were measured 7.12 ± 0.25 mm, whereas, the DP exposed ones were measured from 6.53 ± 0.20 mm to 6.23 ± 0.50 mm.

DP induced oxidative stress

To determine whether DP induced larval oxidative stress, the content of ROS and MDA were quantified at 96 hpf. A significant increase in ROS content was seen in 60 $\mu\text{g/L}$ and 120 $\mu\text{g/L}$ DP treated larvae compare to the control group. Similarly, a significant increase in MDA level was observed in 60 $\mu\text{g/L}$ and 120 $\mu\text{g/L}$ DP treatment groups (Fig. 3).

Comet assay

The DNA single strand breaks were demonstrated as the mean tail DNA content (% tail DNA) induced by different concentrations of DP in common carp embryo at 24 hpf (Fig. 4A). Comparing with the control groups, the common carp embryos in all treatment groups had significant DNA damage. Interestingly, a dosage dependent increase in the mean tail DNA was observed, with higher concentrations of DP lead to much more serious damage. The representative images of DNA damage obtained by the comet assay are shown in Fig. 4B.

Gene expression

The effects of DP on the mRNA levels of four types of embryo developmental genes (*sox2*, *sox19a*, *Mef2c*, and *BMP4*) are shown in Fig. 5. Significant decrease ($P < 0.05$) in the expression of *sox2* gene was observed in the DP exposed embryos of common carp as compared to the control group of embryos at all the time intervals, however, with an increase in both the exposure time, upward trend in *sox2* gene expression (Fig. 5 A). Further, significant increase ($P < 0.05$) in the expression of *sox19a* gene expression were also observed in the DP exposed embryos of common carp as compared to the control group of embryos, presented a trend of increasing first and then decreasing (Fig. 5 B). At the tail-bud formed stage, the *Mef2c* mRNA levels was significantly increased ($P < 0.05$) in the 30 $\mu\text{g/L}$ DP exposed group. However, *Mef2c* expression was inhibited in the 60 and 120 $\mu\text{g/L}$ DP exposed groups compared to the control group. During the hatching period, *Mef2c* mRNA levels increased significantly ($P < 0.05$) in the 120 $\mu\text{g/L}$ treated group, as compared with the control group, while the other treated groups shown no significant change. At the 24 h post-hatching stage, the *Mef2c* mRNA levels were significantly decreased ($P < 0.05$) and significantly increased ($P < 0.05$) in the 30 $\mu\text{g/L}$ treatment group and in the 60 $\mu\text{g/L}$ treatment group respectively, while the 120 $\mu\text{g/L}$ treated group shown no obvious change, as compared with the control group. At the 48 h post-hatching stage, the *Mef2c* mRNA levels were significantly increased ($P < 0.05$) and significantly decreased ($P < 0.05$) in the 30 $\mu\text{g/L}$ treated group and in the 60 $\mu\text{g/L}$ treated group, respectively (Fig. 5 C). At the tail-bud formation stage, compared with the control group, the *BMP4* mRNA levels were significantly increased ($P < 0.05$) and significantly decreased ($P < 0.05$) in the 30 $\mu\text{g/L}$ treated group and in the 60 and 120 $\mu\text{g/L}$ treated groups respectively, as compared with the control group. During the hatching period, the mRNA expression of *BMP4* revealed a significant increase ($P < 0.05$) in the 120 $\mu\text{g/L}$ treated group compared with the control group. However, there are no significant changes in the 30 and 60 $\mu\text{g/L}$ DP exposed groups. *BMP4* expression was significantly increased ($P < 0.05$) in the 60 $\mu\text{g/L}$ treatment group at the 24 h post-hatching stage, while decreased in the 30 and 120 $\mu\text{g/L}$ treated groups in contrast to the control group. Further, there was no obvious induction during the 48 h post-hatching stage (Fig. 5 D).

Relationship between comet assay and morphological abnormality

In order to determine whether there is a relationship between embryo abnormality and DP induced DNA damage, we examined the correlation between DP induced DNA damage and carp embryos morphological distortion (Figure 6). A significant positive correlation was observed between the two parameters.

Discussion

DP acts as a persistent organic pollutants of the aquatic environment that can have harmful effects on aquatic organisms, including fish (OxyChem 2007; Yu et al. 2013; Liang et al., 2014; Sun et al. 2017; Ji et al., 2018). Fish are subjected to low-dose pollutants in water that do not cause death, but cause altered

gene expression or retarded growth (Stěpánov Stanislava, et al. 2012; Chen et al. 2017). However, the effects of DP on common carp development and behind mechanisms have not been studied. Therefore, the present study aim to reveal the effects of the DP exposure on common carp embryo, indicated mortality rate, hatching rate, body length, morphological deformities and DNA single strand breaks and gene expression regulation. This study also provides new data to support the effects of DP on common carp embryos and larvae at relevant environmental concentrations.

Increased mortality, reduced hatchability and hatching delay of DP exposed embryos have been observed in the present study, suggesting the toxicity of DP on common carp embryos. Previous studies have shown similar mortality rate increased in common carp embryos exposed to Cyhalofop-butyl (Xia et al., 2018). Mortalities were also observed in zebrafish embryos exposed to various chemicals or metals such as copper (Frayse et al., 2006; Johnson et al., 2007). It's have been shown that chemically induced changes in mitochondrial bioenergy in zebrafish embryos can lead to abnormal hatching times or tail bending. (Wang et al., 2018; Zhang et al., 2017). Noteworthy is that hatching delay is reported to be associated with the interference in the thyroid hormone system (Timme-Laragy et al., 2006), and DP can disrupts thyroid hormone balance of zebrafish by altering regulatory pathways in the brain (Kang et al., 2016). Thus, we hypothesize that mitochondrial dysfunction and disruptions in TH system induced by DP may contribute to the hatching delay. The present study demonstrated that DP was toxic to the common carp and could affect embryo development and even cause embryo death. Meanwhile, this result confirms the findings described in previous studies such as increased DNA damage and change the expression of related developmental genes.

Signals of apoptosis induced by oxidative stress have proven to be a potential cause of various pathologies (Circu and Aw, 2010). Previous studies suggest that DP exposure induce significant oxidative stress in quail and mice (Li et al., 2013b; Wu et al., 2012). To examine whether DP exposure induces oxidative stress in common carps, the levels of ROS and MDA were measured in 96 hpf larvae. Our results showed that DP exposure to the 60 and 120 µg / L treated groups significantly increased ROS and MDA formation. These results suggested that DP might induce apoptosis through oxidative stress signal pathway, which could be a potential mechanism of observed dysplasia after common carp exposure to DP.

Dysplasia is an important index for evaluating the teratogenicity of pollutants (Niell et al. 2010). Cyhalofopbutyl exposure causes abnormal common carp morphology including yolk sac edema, pericardial edema, deformation of the spine and deformation of the tail (Xia et al. 2018). CPF exposure also resulted in the morphological abnormalities of zebrafish larvae, mainly manifested as pericardial edema and spinal deformities (Jin et al. 2015). Interestingly, in this study we also found a significant reduction in body length of DP exposed common carps. These results are similar with those that reported reduction of fish growth upon exposure to other chemicals, for example organophosphate insecticides and pyrethroid insecticides (Allison, 1977; Machova et al., 2010; Richterova et al., 2015; Werner and Moran, 2008). In the present study, changes in the developmental malformation of common carp larvae may be due to DP exposure induced DNA damage (single/double strand breaks) and oxidative stress. In

addition, the expression of *sox2*, *sox19a*, *Mef2c*, and *Bmp4* gene may involve in the process of morphological deformities.

The comet assay is widely used in genetic toxicity studies (Manas et al., 2009; Nan et al., 2016). The comet assay is an important biomarker for fish genetic toxicity, and DNA strand breaks are measured by the comet assay (Mitchelmore & Chipman, 1998). This study found that DNA single-strand breaks (% tail DNA) in common carp embryos were significantly increased in all treatment groups and strongly correlated with DP doses and exposure time studied indicate the genotoxic potential of DP exposed. These results are similar with the finding of Tasneem and R. Yasmeen (2018) which proved that karanjin can cause DNA damage in common carp detected by comet assay. Since 48–72 hpf is a critical period during the early embryonic development of common carp, considering the effects of nerves and bones on developmental malformations, to further find the damage mechanisms at the level of molecule, the associated genes were investigated in this study. The *sox* gene proteins control a wide range of developmental regulators in the early embryo, has been fairly well characterized regarding its regulatory functions and its expression in embryogenesis. It was identified as an essential factor to activate crystallin genes in the lens cells (Kamachi et al., 1998; Okuda et al., 2010). Studies show that gene expression can be regulated by exposure to chemical genotoxicants, causing DNA damaged of zebrafish (Sandrini et al., 2009; Geffroy et al., 2012, Chen et al., 2019). Damaged genes that cannot be repaired by the DNA repair system may eventually lead to morphological deformities and neurobehavioral defects in zebrafish larvae (Chen et al., 2019). Significant down regulation of *sox2* genes expression in the present study may be due to the sensitivity of the *sox* genes to DP exposure. The significant down-regulation of *sox2* gene expression in this study may be due to the sensitivity of *sox* gene to DP exposure. The up-regulation of *sox19a* at 72 hpf may be crucial in enabling the common carp to cope with oxidative stress and ensure normal development by self-regulation. This result is supported by the increased DNA damage and morphological deformities in the present study. This result provides evidence for increased DNA damage and morphological abnormalities in this study.

Mef2c is one of the key genes involved in regulating cardiac and skeletal muscle development, and can promote skeletal muscle development during embryogenesis (Hinits and Hughes 2007; Molkenin et al. 1995). Research has shown that, this inhibition of BMP signaling during zebrafish fin regeneration was observed to disrupt fin growth, as well as the differentiation and function of nucleated cells (Smith et al. 2006). Our results show that the *Mef2c* mRNA level was significantly decreased in the 30 µg/L treated group at the 24 h post-hatching stage and was significantly decreased in the 60 µg/L treated group at the 48 h post-hatching stage. Compared with the control group, the mRNA expression of *BMP4* in the 60 and 120 µg/L treatment groups was significantly decreased during the tail-bud forming stage. This suggests that DP may negatively affect skeletal muscle development by disrupting the expression of *Mef2c* and *BMP4* genes. The specific mechanism by which this interaction affects skeletal muscle development requires further study.

From the comet assay, it shown that the positive correlation between DNA damage and morphological abnormalities of common carp larvae may indicate that DNA single-strand breaks induce carp embryo

developmental distortion. Similarly, CuInS₂/ZnS quantum dots (QDs) exposure induces impaired growth and DNA damage in fish (Liu et al., 2017). DNA damage may also cause down regulation of related developmental genes, which may be the common cause of all developmental abnormalities observed in the study.

Conclusions

In summary, our results showed that DP inhibits the hatching rate of common carp embryos and even causes the death of common carp embryos. Some successfully hatched embryos were deformed by DP exposure, consistent with significant changes in related genes. So DP has some negative impact on the growth and development of common carp embryos. This study provides a basis for guiding the rational use of DP and promoting the sustainable development of the ecological environment.

Abbreviations

DP:dechlorane plus; C. carpio:Common carp, *Cyprinus carpio* L.; hpf:hours post fertilization; qPCR:quantitative PCR; ROS:reactive oxygen species; MDA:malondialdehyde; DMSO:Dimethyl sulfoxide; DCFH-DA:dichlorofluorescein-diacetate; SEM:standard error of the mean; PE:pericardial edema; BM:body malformations; SC:spinal curvature; QDs:quantum dots.

Declarations

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Authors' contributions

All authors contributed equally to the experiment. All authors participate in the writing of the article.

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Availability of data and materials

The datasets generated and/or analyzed during the current study are not publicly available due to copyright but are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The experimental use of fish was approved by the Animal Ethics Committee of Henan Normal University (HNSD No. 20180901-0915-01).

Consent for publication

Not applicable.

Conflict of interest

The authors have no conflict of interest to declare.

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Table

Table 1 Primer sequences of the genes

Gene name	Primer	Gene ID
sox2	F: 5'- CTCGGGAAACAACCAGAAAA -3'	NM_213118.1
	R: 5'- TCGCTCTCGGACAGAAGTTT-3'	
sox19a	F: 5'- TGCAACAGCAACAACAGCA-3'	NM_130908.1
	R: 5'- GTTGTGCATTTTGGGGTTCT-3'	
Mef2c	F: 5'- GCAGAGACTGTGTGCCATCC-3'	AB012885
	R: 5'- CCTGTGTTTCCTGCACTCG-3'	
BMP4	F: 5'- GGAAGGGAAGAAGAAAGCGT-3'	KP661172
	R: 5'- ACAGGTCCAGCAGATACTG-3'	
β -actin	F: 5'- GCTATGTGGCTCTTGACTTCG-3'	M24113
	R: 5'- CCGTCAGGCAGCTGATAGCT-3'	

Figures

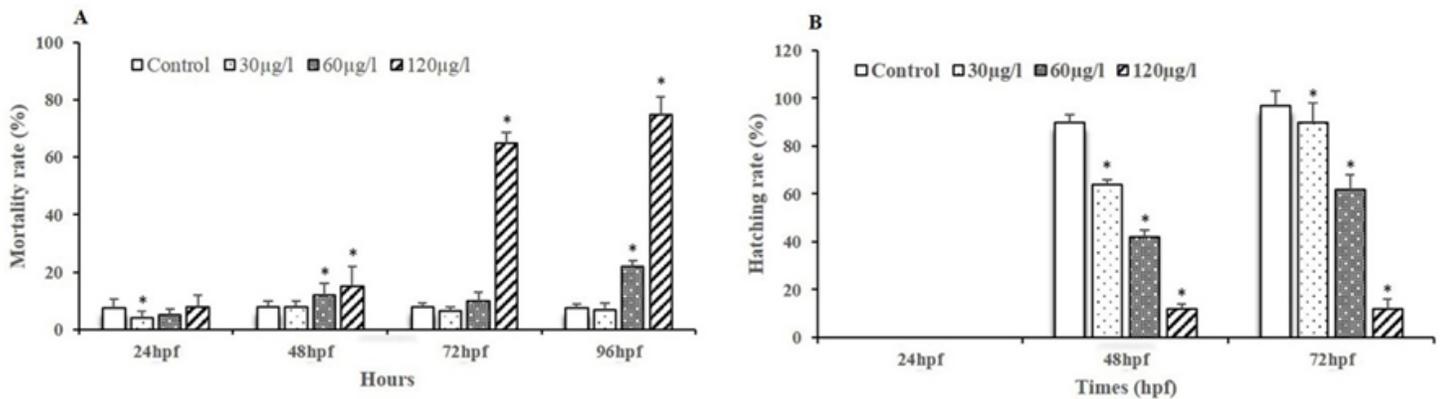


Figure 2

Mortality rate (A) and hatching rate (B) of embryos exposed with different concentrations of DP at different time intervals (Mean \pm SD). * Stands for significant differences among treatments comparing with the control ($P < 0.05$).

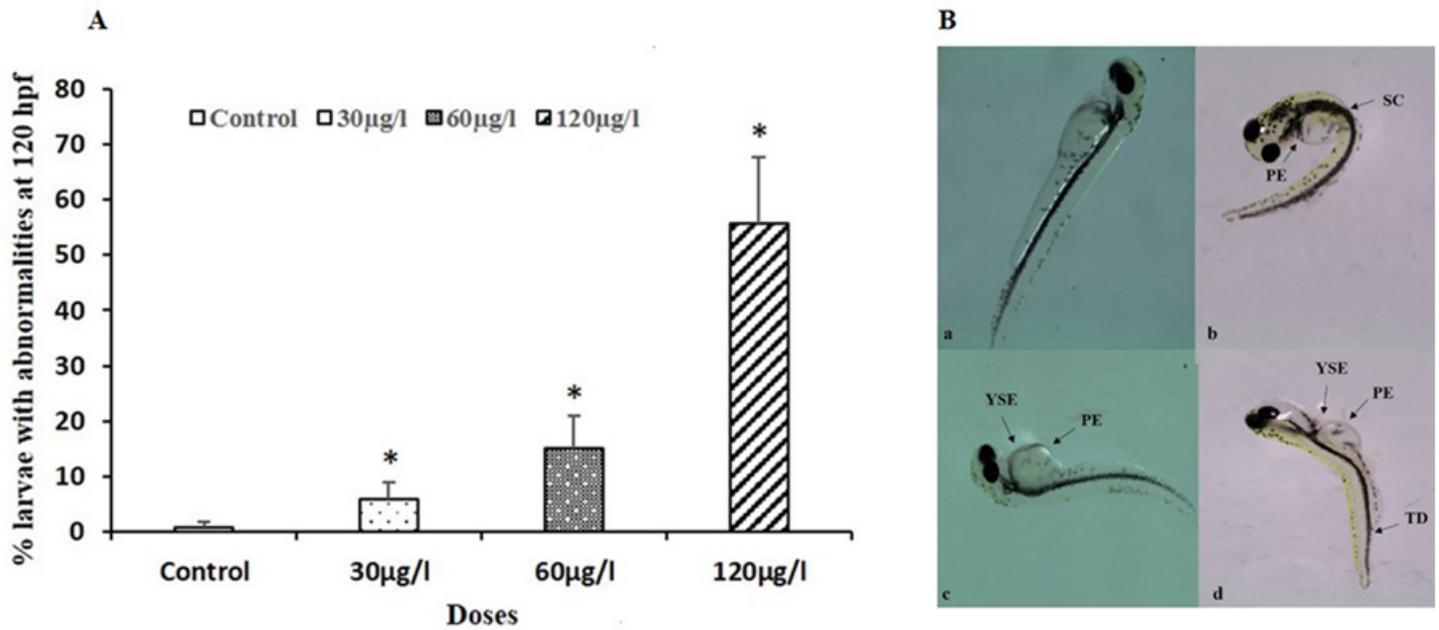


Figure 4

(A) Frequency of morphological deformities (Mean \pm SD) induced by different concentrations of DP exposed in embryos at 120 hpf. * Stands for significant differences among treatments comparing with the control ($P < 0.05$). (B) Morphological effects of DP on larvae stages. a Normal larvae. b Larvae with spinal curvature (SC) and pericardial edema (PE). c Larvae with yolk sac edema (YSE) and pericardial edema (PE). d Larvae with pericardial edema (PE), yolk sac edema (YSE), and tail deformation (TD).

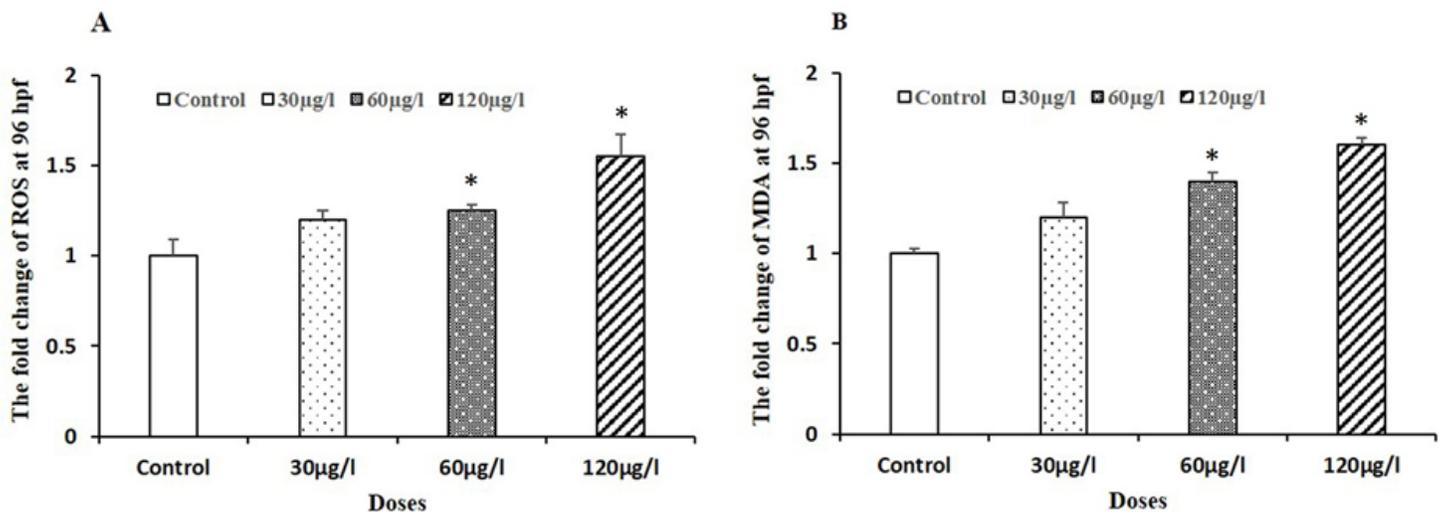


Figure 6

DP exposure induced oxidative stress. DP exposure increased levels of ROS (A) and MDA (B) formation (20 larvae each replicate, $n = 6$ replicates) in common carp at 96 hpf. Data are (Mean \pm S.D). * Stands for significant differences among treatments comparing with the control ($P < 0.05$).

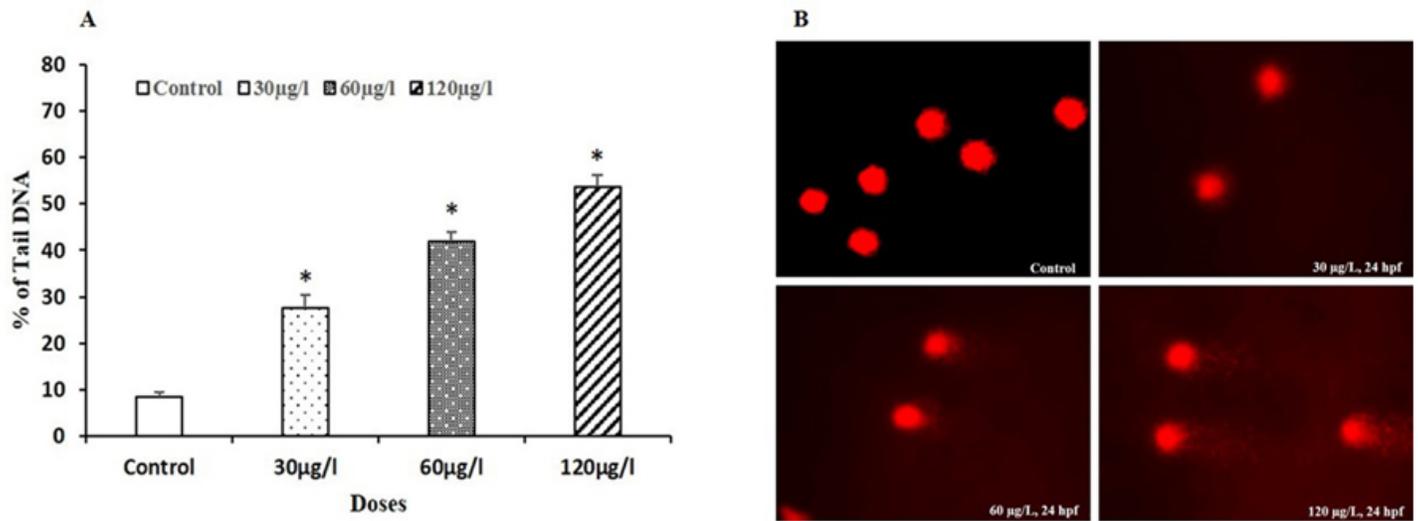


Figure 8

(A) DNA Damage (% of tail DNA) in embryos exposed with different concentrations at 24 hpf after exposure. Data are (Mean \pm SD). * Stands for significant differences among treatments comparing with the control ($P < 0.05$). (B) Representative epi-fluorescence images of the comet assay to embryos cells showing the genotoxic effect of DP.

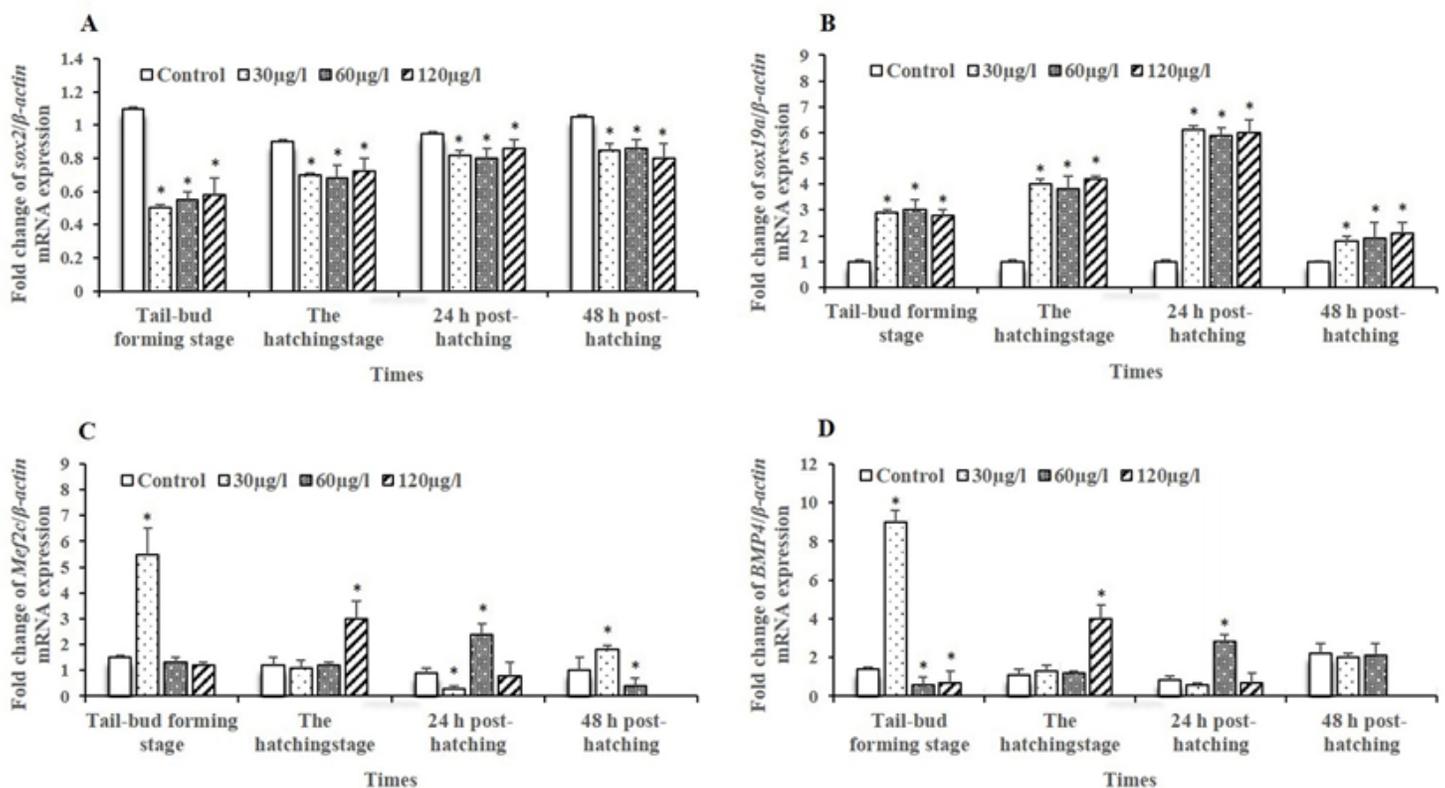


Figure 9

Effect of DP exposure on *sox2* *sox19a* *Mef2c* and *Bmp4* gene expression in embryos. Data are (Mean \pm S.D). * Stands for significant differences among treatments comparing with the control ($P < 0.05$).

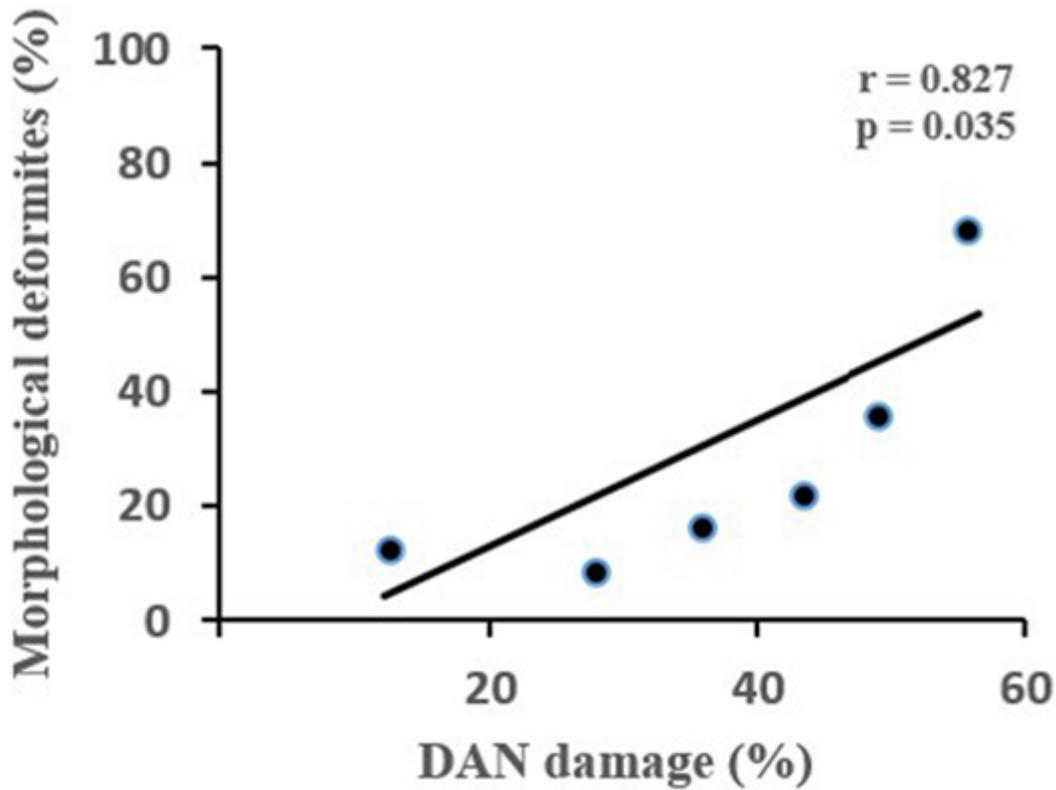


Figure 12

Correlation between DNA damage and morphological deformities in common carp Pearson correlation was used and the level of significance was set at 95%.

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