Saudi Journal of Biological Sciences 28 (2021) 4738–4750

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Bisphenol A induced toxicity in blood cells of freshwater fish *Channa punctatus* after acute exposure

Prince Sharma, Pooja Chadha *

Department of Zoology, Guru Nanak Dev University, Amritsar, Punjab 143005, India

ARTICLE INFO

Article history:
Received 12 March 2021
Revised 26 April 2021
Accepted 27 April 2021
Available online 5 May 2021

Keywords:
Bisphenol A
Oxidative stress
Antioxidant enzymes
Genotoxic
Channa punctatus
Scanning Electron Microscopy

ABSTRACT

The widespread use of bisphenol A (BPA) has led to its ubiquity in the natural environment. It is extensively incorporated into different industrial products and is associated with deleterious health effects on both public and wildlife. The current trial was conducted to determine the toxic potential of bisphenol A using various parameters viz haematological, biochemical, and cytological in freshwater fish *Channa punctatus*. For this purpose, fish were exposed to 1.81 mg/l (1/4 of LC_{50}) and 3.81 mg/l (1/2 of LC_{50}) of BPA along with positive (acetone) and negative controls (water) for 96 h. The blood samples were collected at 24, 48, 72, and 96 h post-exposure. Compared to the control group, fish after acute exposure to BPA showed a significant decrease in HB content, number of red blood cells, PCV values whereas a significant increase in WBCs count was recorded with an increase in the exposure period. Besides, oxidative stress (determined as malondialdehyde content) increased as BPA concentration increased. Further, the activity of different antioxidant enzymes like catalase, and superoxide dismutase decreased significantly after treatment. Results also showed significantly increased frequency of morphological alterations, nuclear changes, and increased DNA damage potential of BPA in red blood cells. Further structural analysis of erythrocytes in maximally damaged group using Scanning Electron Microscopy was performed. The study concludes that BPA exhibits genotoxic activity and oxidative stress could be one of the mechanisms leading to genetic toxicity.

1. Introduction

Throughout the world, attention towards the monitoring of potentially harmful effects of environmental contaminants including pesticides, herbicides, and industrial effluents has dramatically increased (Ghaffar, 2020). Industrial effluents from different industries such as textile mills, chemical manufacturing, pharmaceuticals, plastic industry, paper industry, etc usually add several contaminants like pesticides/insecticides, heavy metals, flame retardants, plasticizers, etc to the water bodies.

Many of these synthetic compounds are endocrine disruptive (Scarano et al., 2019; Xu et al., 2018; Zhou et al., 2019) and cause multiple organ dysfunctions in animals including fish (Ghaffar et al., 2018; Naz et al., 2020). These endocrine disruptors tend to bioaccumulate and thus alter the hormone system of both humans as well as wild animals and can be hazardous even at very low concentrations (Vieira et al., 2020). EDCs are potential mimics of some naturally occurring hormones like androgens and estrogens. They exert their toxicity by interfering with hormonal homeostatic mechanisms that are involved in the growth and development of various tissues (Cargnelutti et al., 2020). Among different endocrine-disrupting chemicals (EDCs), bisphenol A is a well-known endocrine-disrupting compound that is reported to induce adverse effects on the pituitary (Chen et al., 2017), gonads (Wang et al., 2019), and brain (Cano-Nicolau et al., 2016; Molina et al., 2018).

Bisphenol A, an endocrine-disrupting organic and synthetic compound, is widely used in various dietary consumer products, canned food products, and plastic food packaging due to its ability to protect against corrosion. Not only this, but it has also found its importance in the packaging of food items such as baking powder, cereals, and yeast (Bowes and Halden, 2019). BPA has been used as an additive in polycarbonate plastics and epoxy resins to harden the products for their usage as water bottles and food storage containers, due to its cross-linking properties (Darbre, 2020). It's important properties include low vapor pressure, moderate water

* Corresponding author.
E-mail address: poojachadha77@yahoo.co.in (P. Chadha).

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2021.04.088
1319-562X/

solubility, and low volatility. It is solid at room temperature (TSAI, 2006). It contains two functional phenol groups that allow the chemical to interact with estrogen and androgen receptors as both an agonist and antagonist (Michałowicz, 2014).

According to recent reports by the environment protection agency (EPA) more than one billion pounds of BPA leaks into the environment every year, thus illustrating the extent of potential exposure problems that can be faced (Shafei et al., 2018a). (Porreca et al., 2016) reported that BPA exposure can be associated with an increased risk of carcinogenesis and an increase in the sensitivity of certain cell types. Recently, BPA has also been shown to cause genotoxicity either directly or indirectly in various *in vitro* systems (Jalal et al., 2018).

Bisphenol A is suspected to be related to many health implications such as uterine cancer, low sex-specific neurodevelopment, neurotoxicity, immune toxicity, and interference of cellular pathways (Shafei et al., 2018b; Watkins et al., 2017). BPA has aromatase-like activity and can convert testosterone to estrogen to activate the aryl hydrocarbon receptor which is involved in the synthesis and degradation of steroid hormones (Bonefeld-Jørgensen et al., 2007). Generally, BPA could impair the normal reproductive function by disturbing the sex hormone excretion and activity, and also induce the occurrence of infertility. These BPA–estrogen receptor interactions have been considered as possible explanations underlying few epidemiological associations between estrogen activity and asthma (Bonds and Midoro-Horiuti, 2013; Thornton et al., 2012). BPA can exert detrimental effects on the male reproductive system and is also reported to cause prostate cancer (Cariati et al., 2020). Sperm quality in the men was also reported to be affected by BPA exposure (Li et al., 2011).

Gascon et al. (2015) reported that prenatal BPA exposure might also affect the normal respiratory function and also showed a marked effect on the increased level of asthma symptoms and a high incidence rate of respiratory infections occurring throughout childhood. BPA exposures were also shown to be negatively associated with the general immune response while studying a strong cross-sectional study of adults and children (age 6 and above). BPA has proved to be a potent chemical that can disrupt the immune function and also promote the occurrence of some of the known allergy-related diseases such as bronchial hyper-responsiveness, asthma, diabetes mellitus type 1 (T1D), and autoimmune diseases (Donohue et al., 2013; Zhou et al., 2017). BPA has been known to play a significant role in the development of diabetes, cardiovascular disease (CVD), and obesity also. The structure of BPA is similar to 17β- estradiol and thus show binding to estrogen-related (ER) receptors such as ERα, ERβ, ERγ, peroxisome proliferator-activated receptor gamma (PPAR-γ) and G-protein coupled estrogen receptor GPR30 (Fenichel et al., 2013; Delfosse et al., 2014).

Because of human health and ecotoxicology risk, numbers of countries have forbidden the use of various products with BPA addition. It is frequently being detected in the environment and has become a serious health issue due to its presence in food organisms and drinking water (Buha Djordjevic et al., 2020; Pal and Reddy, 2018).

Fish have been considered as an efficient and effective model to evaluate the toxic, mutagenic, and carcinogenic potential of pollutants (Monteiro et al., 2010). They are the extreme inhabitants of all zones of aquatic habitat, respond to mutagens at low concentration, bioaccumulate the environmental pollutants, and provide early warning for pollution-induced environmental changes.

In vivo toxicity reports due to bisphenol A exposure is very limited. So the present investigation was undertaken to determine the LC_{50} value of BPA for freshwater fish *C. punctatus*. Further, the effect of sublethal concentration was studied on behaviour, haematological, biochemical as well as cytological parameters.

2. Materials and methods

2.1. Experimental organism and chemicals

Freshwater air-breathing food fish *C. punctatus* were purchased from the local market with average weight (25–30 g) and length (11–13 cm). Fishes were transported to the animal house to big glass aquaria (dimensions: 60 × 60 × 60 cm). 0.2% potassium permanganate ($KMNO_4$) was used as a prophylactic measure for any kind of dermal infection. Fish were acclimatized for two weeks. They were fed with boiled Chicken eggs. Water was changed on alternate days and any kind of debris was removed daily to remove unutilized feed and to reduce ammonia content. Only healthy and active fishes were used for the experiment after acclimatization. The weight and length of fishes were noted.

BPA used in the present study was obtained from Sigma Aldrich (CAS 80-05-7 and purity 99%). The stock solution of BPA was prepared in acetone as BPA is less soluble in water. For this 1 mg BPA was dissolved in 10 μl of acetone.

2.2. Water parameters

Water parameters were analyzed by a water analysis kit. The values of different water parameters analyzed during the experiment are: temperature 27.5 °C, pH 7.6; dissolved oxygen (DO) 6.3 mg/l, total dissolved solids (TDS) 0.29 g/l, hardness 244 mg/l, and conductivity 502 μs/cm.

2.3. LC_{50} determination and behavioural studies

The percentage mortality of fish in different concentrations of BPA was determined at 96 h of exposure. A range-finding test was conducted and then the appropriate concentration range was selected. Further, the experimental fishes were apportioned into batches of 10 each and were subjected to discrete concentrations of BPA ranging from 4 mg/l to 12 mg/l. Mortality of fish was recorded at 24, 48, 72, and 96 h of exposure with BPA. The test chemical was renewed daily during the experimental period to maintain the dissolved oxygen concentration at an optimum level. The experiment was implemented in duplicates and repeated three times to validate the results. The median lethal concentration was calculated by using computer software Probit Analysis (Finney, 1971). The percentage mortality of *C. punctatus* was observed to be 0% and 100% at 4 mg/l and 12 mg/l concentration of BPA respectively and LC_{50} value was calculated. During LC_{50} determination behaviour alterations were also noted.

2.4. Exposure to sublethal concentrations and experimental setup

After determining LC_{50}, 2 sub-lethal doses were selected, i.e. 1.81 mg/l (one-fourth of LC_{50}) and 3.81 mg/l (one-half of LC_{50}). Two controls were taken: one was maintained in tap water (negative control) and another (positive control) was treated with acetone in equal amount (μl/l) to that delivered in the experimental tanks tested. The experiments were carried out for 96 h duration to reveal acute effects. Haematological parameters were assessed to evaluate alterations in erythrocytes. Oxidative damage was noticed by estimating malondialdehyde (MDA) levels and activities of antioxidant enzymes like catalase (CAT) and superoxide dismutase (SOD). Genotoxicity was determined using micronuclei assay and comet assay. Morphological alterations in RBCs were studied by SEM.

2.5. Haematological parameters

Haematological parameters were assessed by the standard technique suggested by Jain (1986). Present analysis manifests the correspondence of blood parameters like Haemoglobin (Hb), red blood cells (RBĆs) count, white blood cells (WBĆs) count, and packed cell volume (PCV). Blood was taken in EDTA-coated Eppendorf tubes by puncturing the heart of the fish. Estimation of haemoglobin was deciphered by using Sahlís haemoglobinometer. Neubauer haemocytometer is used for red blood cell counting. Hayems fluid is used in 1:200 proportions to dilute the blood. Total number of erythrocytes was counted per 10^6 mm^3. Hematocrit levels (PCV) were ascertained by siphoning fresh blood into microhaematocrit tubes and then allowed to centrifuge (Microcentrifuge, Remi motors, Bombay, India) for 5 min at 9000*g*.

2.6. Lipid peroxidation

Malondialdehyde (MDA) activity was estimated according to Ohkawa et al. (1979) with slight modifications. Blood sample of around two milliliters was allowed to clot for 2 h at room temperature and then centrifuged for 10 min at 3000 rpm. 1 ml of thiobarbituric acid (0.6%) and 1 ml of trichloroacetic acid (17.5%) were added to 1 ml of serum, this preparation was then put in a boiling water bath for 30 min. 1 ml of n-butyl alcohol was annexed to test tubes after cooling, and then again centrifuged for 10 min at 3000 rpm. Sample absorbance was computed at 532 nm.

2.7. Antioxidant enzymes

Catalase (CAT) activity was measured according to the method of Aebi (1984) with some modifications. The reaction mixture containing 0.8 ml phosphate buffer (50 mM, pH 7.0), 0.l ml sample and 0.1 ml Triton X-100 (0.2%) then incubated at room temperature for 10 min. The reaction was initiated by the addition of 2.0 ml H_2O_2 (0.03 M prepared in potassium phosphate buffer, pH 7.0), and absorbance change per min was recorded at 240 nm. Superoxide dismutase was estimated according to the method given by Kono (1978). The enzyme extract (0.5 ml) was added to 1.3 ml of 50 mM Sodium carbonate buffer (pH10.0), 0.5 ml of NBT, 0.1 ml of 0.6% Triton X-100 and 0.1 ml of 20 mM Hydroxylamine hydrochloride (pH 6.0) in a cuvette. The change in absorbance was observed at 540 nm for 5 min at 25○C- 27○C room temperature.

2.8. Micronuclei assay

Blood samples were collected, and slides were prepared by smearing one drop of blood on a clean microscopic slide. For the fixation of smear, methanol was used, slides were kept in methanol for 10–15 min and then left to air dry at room temperature. Slides were stained with 6% Giemsa for 20 min. For each group (treated as well as control) 1000 erythrocytes were examined for the presence of micronucleated (MNC) and aberrant cells (AC) under a binocular microscope (Olympus), using oil immersion lens 100 X. All the nuclear and cytoplasmic abnormalities are collectively considered as AC (Kumar et al., 2010). Nuclear abnormalities include notched, lobed, deformed, blebbed, and elongated nuclei, Cytoplasmic abnormalities include: Karyolysis, vacuolated cytoplasm, and swelled cells.

2.9. Single-cell gel electrophoresis (Comet assay)

Alkaline version of Comet assay was performed using blood samples (Ahuja and Saran, 1999) by puncturing the heart, 10 μl of blood is immediately diluted in 1 ml of phosphate buffer saline. Microscope slides were coated with 1% of normal melting point agarose (NMPA), and then the slides were incubated at 37 °C, overnight. 0.5% of low melting point agarose (LMPA) mixed with 20 μl of blood was added to the coated slides. The slides were then kept in the refrigerator at 4C for 10–15 min. Third layer of 0.5% LMPA was poured on the slides and returned to the refrigerator at 4 °C for another 10 min. Slides were then placed in lysis buffer for about 3 h in the refrigerator. After the lysing step the slides were incubated in electrophoretic buffer followed by electrophoresis for 20 min at 300 mA and 24 V. Neutralization buffer was used to neutralize the slides for 15 min. Slides were kept for drying. Ethidium bromide was used to stain the slides. Blotting paper was used to remove an excess of stain, Fluorescent microscope with 40X magnification, excitation filter of 515–560 nm, 590 nm barrier filters was used for scoring of slides. CASP LAB image analysis software was used for examining the comets.

2.10. Scanning electron microscopy

Blood was collected from both control and exposed fish. Three to four drops of blood were immediately fixed in 2.5% glutaraldehyde prepared in 0.1 M phosphate buffer (pH 7.4) for 2–3 h and the sample was centrifuged at 1500 rpm for 5 min. After discarding the supernatant, the erythrocyte pellet was washed 2–3 times with phosphate buffer. The pellet was then suspended in a small volume of distilled water and dehydrated with increasing concentrations of ethanol. A small drop of erythrocyte suspension was applied to a coverslip (circular, 10 mm). Air-dried samples were sputter-coated with gold and examined under a scanning electron microscope at an accelerating voltage of 15–20 kV.

2.11. Statistical analysis

Statistical analysis was performed using a software program (SPSS 16.0 for windows). All data were presented as mean ± SE. One-way ANOVA followed by posthoc Tukey's test was used to assess the significance of the difference between control and treatment groups. P values less or equal to 0.05 were considered statistically significant.

3. Results

The 96 h LC_{50} value of BPA was determined to be 7.625 mg/l for *Channa punctatus* (Table 1). The disparities in behaviour of *C. punctatus* after the treatment of BPA are presented in Table 2. At the start of the exposure, fishes showed vigilance and ceased swimming, hyperexcitability, and erratic movements were observed with increasing concentrations. Copious amount of mucus is secreted from the whole body. High pigmentation, loss in the equilibrium, darting movements, and loss of Schooling behavior were also noted. Fishes were seen engulfing air through the mouth and high opercular activity was observed. Eventually fish died and a change in color of the gill lamellae and coagulation of mucus was seen in dead fishes. The behaviour alterations were found to be more pronounced after administration with higher concentrations than lesser concentrations as depicted in Fig. 1.

The toxic effect of BPA was perceived by studying hematological parameters, oxidative stress, and DNA damage in blood cells of fish at different time durations for both concentrations. Acute effects were examined up to 96 h. Results are put forth in Figs. 2–7. In the present study sublethal exposure of BPA for 96 h effectuated notable alterations in haematological parameters Fig. (2a-d). After 96 h of exposure Hb content (gm), RBC count (10^6/mm^3), and packed cell volume (%) showed a significant declining trend in comparison with negative as well as a positive control. Hb content

Table 1
Determination of LC_{50} value of BPA in *C. punctatus* for 96 h.

Chemical	Fish	Upper limit	Lower limit	LC_{50}
BPA	*C. punctatus*	9.138	6.267	7.625 mg/l

Table 2
Impact of BPA on behaviour of fish *Channa punctatus* after treatment for 96 h.

Parameters	Control	4 mg/l	5 mg/l	7 mg/l	9 mg/l	10 mg/l	12 mg/l
Hyperactivity	–	+	++	++	+++	+++	+++
Loss of balance	–	+	++	++	+++	+++	+++
Rate of Swimming	+	+	+	++	++	+++	+++
Rate of Operculum activity	+	++	++	++	++	+++	+++
Pigmentation	–	++	++	+++	+++	+++	+++
Mucus secretion	–	+	++	++	+++	+++	+++

(–) None, (+) mild, (++) Moderate, (+++) Strong.

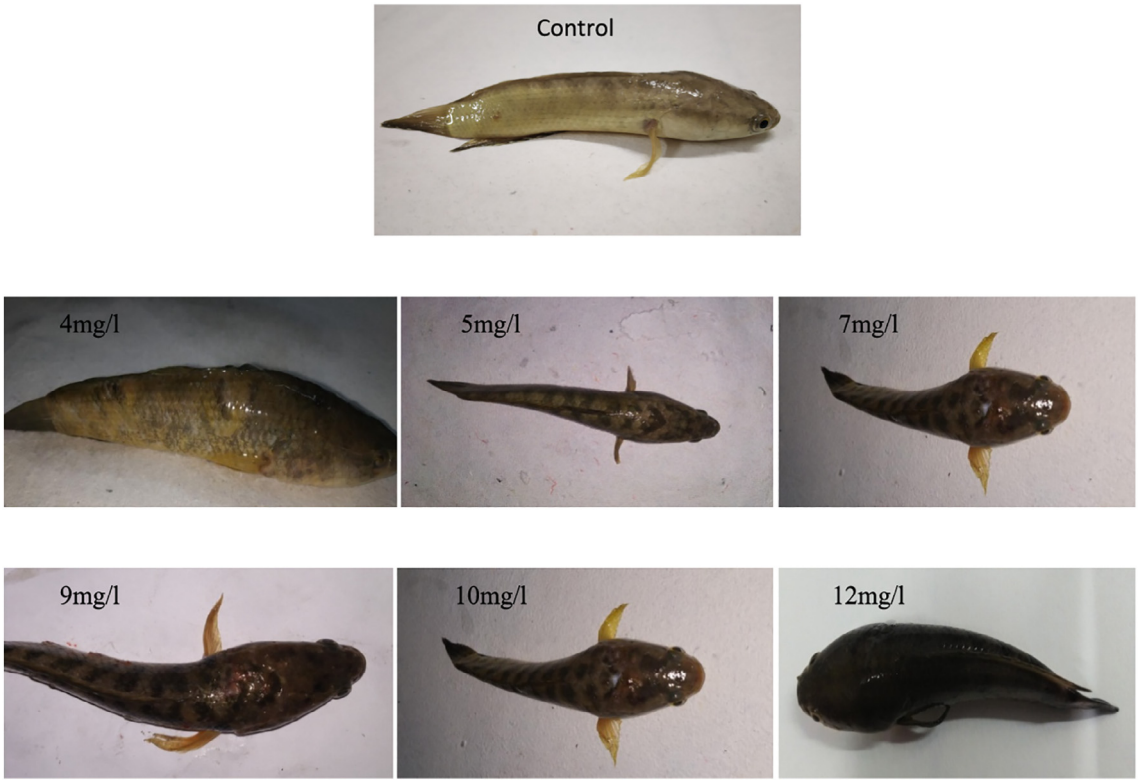

Fig. 1. Pigmentation in fish *C. punctatus* exposed to different concentrations of BPA.

explicates dose-dependent significant decline with an increase in time duration. The lowest levels of Hb were found at 96 h of exposure with the highest concentration (3.81 mg/l). The value decreases from 10.46 ± 0.12 (control) to 6.43 ± 0.23, which is 38.53% less than the control value. Similarly, a significant decrease was perceived in RBC and PCV levels. RBC count decreased from 2.23 ± 0.04 (Control) to 1.27 ± 0.02 after 96 h of exposure. PCV level decreases from 42.15% (control) to 27.19% (3.81 mg/l). THE highest WBC count ($10^3/mm^3$) was observed at 96 h of exposure and the increase was significant with BPA treatments along with time duration. Values of WBC count showed a significant hike which is 1.31 folds as compared to the control group.

Fig. 3a displays the effect of BPA on MDA levels in blood of fish *C. punctatus*. For acute exposure, results were found to be significantly different in comparison with the controls for 24 h, 48 h, 72 h, 96 h, of exposure for both the concentrations of BPA. MDA levels showed a significant hike at 96 h which was found to be 4.20 and 3.10 folds higher than negative control after administration of 1.81 mg/l and 3.81 mg/l BPA respectively. CAT activity was significantly ($p < 0.05$) decreased in a concentration and time-dependent manner in blood after exposure of sub-lethal concentrations when compared to the control group (Fig. 3b). The exposure of fish to BPA resulted in the reduction of SOD activity as compared to that of the control specimen and the results are shown in Fig. 3c. A significant ($p < 0.05$) decrease in SOD activity was observed after 24, 48, 72, and 96 h exposure in blood at both concentrations as compared to control. The maximum decrease in CAT and SOD activity was observed after 96 h with the highest concentration.

Micronucleated (MNC) and aberrant cell (AC) frequency in peripheral blood circulation is given in Fig. 4(a-b). The present study discloses the significant increase in MNC and AC frequency after treatment with BPA as compared to the control groups. In the negative control group, MNC and AC frequency was very less

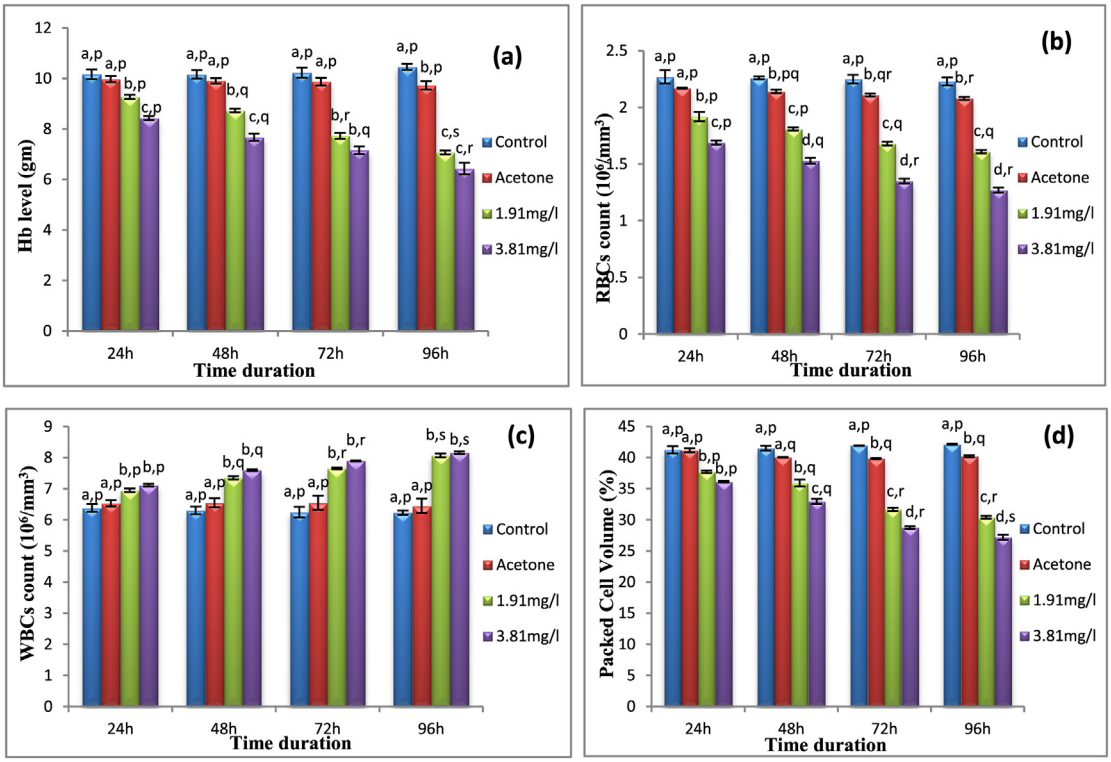

Fig. 2. (a-d):- Haemoglobin concentration (gram), Red blood cell count (X 10^6/mm^3), White blood cell count (X 10^3/mm^3), and Packed cell volume (PCV %) in *fish C. punctatus* exposed to different concentrations of BPA for different hours. Different letters a, b, c, d signify the effect of treatment at the same time interval, and p, q, r, s signify the effect of duration of exposure.

whereas in the positive control slight non-significant increase was noticed. A significant dose-dependent increase was observed for both concentrations. When exposed to 3.81 mg/l BPA, the percentage of micronucleated cell raised from 0.1 ± 0.05 to 0.81 ± 0.01, and the percentage of aberrant cells increased from 7.68 ± 0.17 to 43.89 ± 1.24 (Mean ± S.E.). Thus significant effects ($P < 0.05$) of concentrations and time of exposure were revealed in the results.

Further DNA damage was assessed using comet assay by investigating tail length and tail moment as parameters. The impact of treatment of BPA on the tail length and tail moment on blood cells of *C. punctatus* is given in Fig. 5(a-b). The negative control group exhibited the lowest DNA damage. A slight hike in the value was perceived in the acetone-treated control groups but the increase was non-significant. Treatment with both the concentrations of BPA actuated significant change for both the parameters as compared to both the control groups (Tukey's test). Maximum damage was observed at 24 h of exposure with the highest concentration of BPA (3.81 mg/l) where tail length increases 2.07 times and tail moment increases 3.45 times in comparison with control. At 48 h there was a decrease in the value for both the parameters and at 72 and 96 h again an increase in the value was observed. The effect of duration of exposure was also found to be significant (One way ANOVA, $p < 0.05$).

Fig. 8 depicts scanning electron micrographs of blood cells of control as well as exposed group. RBCs of the control group were elliptical whereas erythrocytes from the exposed group showed various abnormalities such as irregular shaped, cytoplasmic bleb, notched, elongated cells, and fused cells.

4. Discussion

Bisphenol-A is one of the most studied endocrine-chemicals, which is widely used all over the world in plastic manufacture. Because of its extensive use and continuous discharge from industries, it has become the most important and deleterious health threat for both terrestrial and aquatic life (Andújar et al., 2019; Yang et al., 2019).

In the present study, the LC_{50} value for BPA was calculated to be 7.625 mg/l (Table 1). The LC_{50} value of BPA for freshwater fish is scarcely explored. LC_{50} of BPA previously determined for *Daphnia magna* and *Oryzias latipes* was found to be 11.7 mg/l and 5.0 mg/l respectively (Li et al., 2017). While 96 h LC_{50} for Cichlid fish, *Etroplus maculatius* exposed to BPA was found to be 6.48 mg/l (Asifa and Chitra, 2015). LC_{50} values determined for different animals were found to be varied due to the variations in tolerance levels among different organisms.

An organism's first response to environmental change is frequent alterations in its behavior (Nagelkerken and Munday, 2016). Behaviour is the result of several complex processes of development and physiology (Wong and Candolin, 2015) and therefore provides a comprehensive measure of exposure to multiple stressors. Behavior is the result of adaptation to a changing environment and allows the organism to adjust internal and external stimuli to cope with the variable environment (Sharma et al., 2019). It is a sensitive measure of an organism's response to stress including environmental contaminants.

In our trial results exhibited different clinical and behavioral ailments like hypersecretion of mucus from gills, mouth, loss of equilibrium, erratic movement, gulping of air, loss of coordination, and lying on one side in fish at low concentrations. Similar to our findings, (Akram et al., 2021) also observed loss of equilibrium, erratic movement, secretion of mucus, etc in *Aristichthys nobils* exposed to BPA. Previously, jerky movement and erratic swimming due to BPA at low concentrations in *Labeo rohita* (Faheem et al., 2017) and loss of balance, erratic swimming, and altered swimming pattern in *Ctenopharyngodon* (Kaliappan Krishnapriya et al., 2017) have been observed. (Saili et al., 2012) observed BPA-induced hyperactivity in

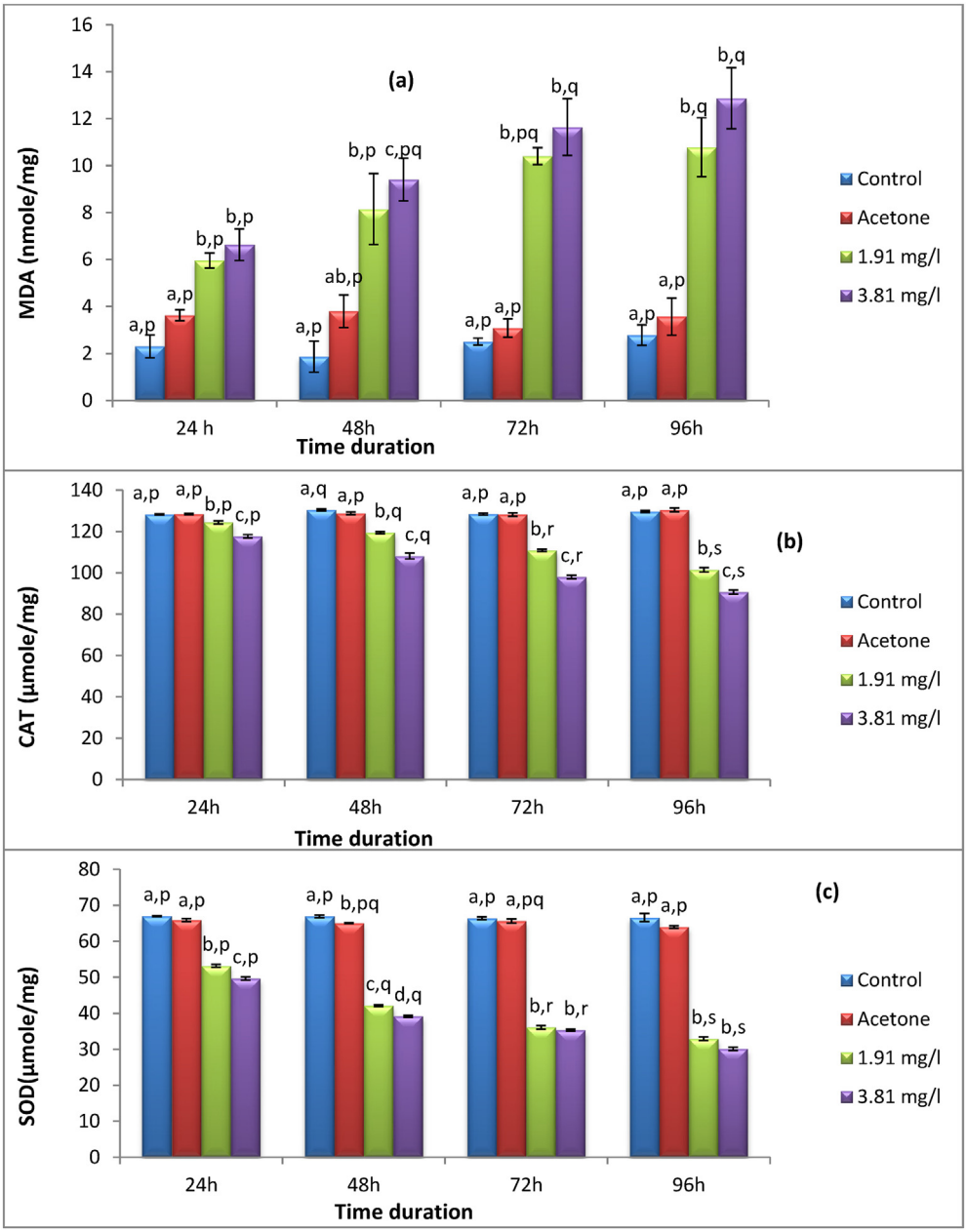

Fig. 3. Effect of different concentrations of BPA on (a) MDA level, (b) CAT and (c) SOD activity in the blood of *C. punctatus* at different hours of exposure. Error bars represent standard errors (SE). Different letters a, b, c, d signify the effect of treatment at the same time interval, and p, q, r, s signify the effect of duration of exposure.

larval fish and learning deficits in adult zebrafish. (Wang et al., 2013) reported that BPA exposure had altered spontaneous movement, decreased tactile response, and swimming speed in response to light stimulation by inducing axial muscle damage in larval zebrafish.

The abnormal behaviors observed in the fish may be caused by the neurotoxic effects and also by the irritation to the perceptive system of the body. Toxicants may damage nerve cell bodies, axons, and myelin sheaths. At the biochemical level, they can alter the synthesis and release of neurotransmitters, which may be associated with behavioral changes (Sharma et al., 2019).

With an increase in the amount of dose, the hyperventilation, hyperexcitability, and loss of equilibrium can be seen in fishes. It may occur due to the effect of BPA on fish's central nervous system. Many authors have suggested that hyperexcitability in fishes is due to the hindrance in the activity of the enzyme acetylcholine esterase (AChE) (Mckenzie et al., 2009). Under the influence of BPA fish demonstrated surfacing phenomenon and loss of equilibrium. Similar results were elicited by (Mishra and Mohanty, 2008) in fish *C. punctatus* subjected to chromium. After the exposure, a plenteous amount of mucus exudates from the body of *C. punctatus.* The secretion of excessive mucus is probably due to irritation of the skin due to direct contact with the toxicant. Mucus forms a layer between the body and toxicant to minimize irritating effect (Rao, 2006) and also inhibits the diffusion of oxygen during the gaseous exchange (KUmar et al., 2015). Similar outcomes were reported in *Orechromis niloticus* (Benli and Koksal, 2005) larvae and fingerlings exposed to ammonia, and in *Clarias gariepinus* (Adedeji et al., 2008) subjected to diazinon.

Blood is a pathophysiological reflector of the body because it is highly susceptible to internal and external environmental fluctuations. Blood parameters are contemplated as a sensitive measure of

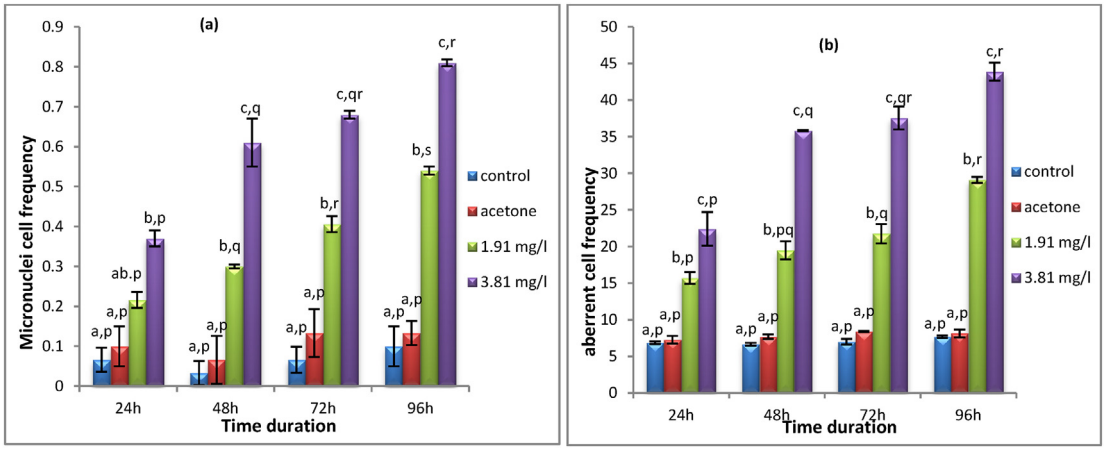

Fig. 4. Effect of different concentrations of BPA on (a) micronuclei cell frequency and (b) aberrant cell frequency in blood cells of *C. punctatus* at different durations of exposure. Error bars represent standard errors (SE). Different letters a, b, c, signify the effect of treatment at the same time interval, and p, q, r, s signify the effect of duration of exposure.

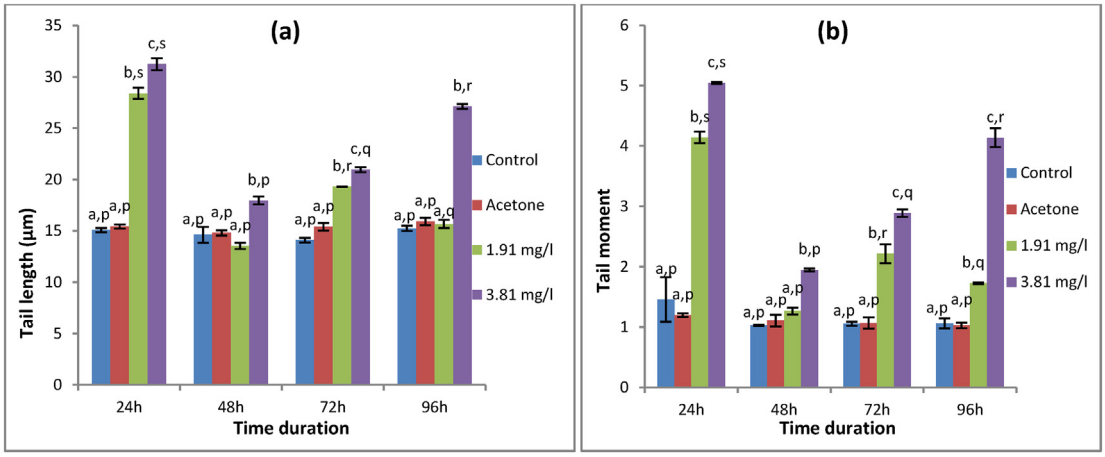

Fig. 5. Effect of different concentrations of BPA on (a) tail length and (b) tail moment in blood at different durations of exposure. Error bars represent standard errors (SE). Different letters a, b, c, signify the effect of treatment at the same time interval, and p, q, r, s signify the effect of duration of exposure.

stress in fish exposed to different water pollutants and toxicants like industrial effluents, metals, pesticides, chemicals, biocides, etc. The current study divulges that exposure to sublethal concentrations of BPA up to 96 h inflicts significant changes in haematological parameters in fish *C. punctatus.* Diminution in hemoglobin content, RBC count, Packed cell volume was discerned in the outgoing study Alleviation in WBC count was also perceived. (Sharma and Chadha, 2015) asserted similar outcomes in *C. punctatus* subjected to sublethal concentrations of nonylphenol. Similar results were observed by (Parveen et al., 2017) in *C. punctatus* exposed to tannery effluent. Exposure to BPA resulted in decreased Hb content, RBCs count, and increased WBCs count in *Hetreropneustes fossilis* (Aiswarya and James, 2016). Lower RBC count and Hb levels were also examined in grass carp *Ctenopharynodon idellus* injected with ammonium acetate (Xing et al., 2016). Attenuation in erythrocyte number may be due to the fragmentation of erythrocytes cell membrane or due to the disarray in hemopoiesis due to disruption in spleen and kidney by toxicants (Banaee, 2013). The prime purpose of WBC count or total leukocyte count (TLC) is to contend against infections, shield the body against incursions by extraneous substances, and induce as well as disseminate antibodies (Ajima et al., 2015). The increase in WBCs count observed in the present study could be attributed to a stimulation of the immune system in response to tissue damage caused by BPA.

Role played by ROS in cell survival, apoptosis, and death has been depicted by (Franklin, 2011). In the experimental studies by (Abdollahi et al., 2004; Abhilash and Singh, 2009) it was concluded that oxidative stress in biological systems instigates as a consequence of a discrepancy between the production of oxidizing species and cellular antioxidant defenses. Measurement of alterations in the levels of GST, CAT, and SOD has been considered effective to assess the overall antioxidant status of an organism. It depicts the overall health of an organism, as any decrease in their activity indicates poor detoxification capacity.

The present study shows that there is a significant difference between controls and treatment groups regarding the MDA content, CAT, and SOD activities after acute exposure. After treatment with BPA, the highest MDA content was seen in the 96 h exposure group in the blood where the value increased 4.63 folds. The assault of lipid peroxides on polyunsaturated fatty acids of cell membranes can be a potential explanation for the rise in MDA due to BPA intoxication. This affects the biophysical properties because of the cross-linking of membrane components. As a consequence, it contributes to decreased membrane stability, increased membrane permeability, inactivation of enzyme activity, and loss of essential fatty acids. Lipid hydroperoxides generated during lipid peroxidation also cause DNA damage by inducing single and double-strand breaks in DNA (Ayala et al., 2014).

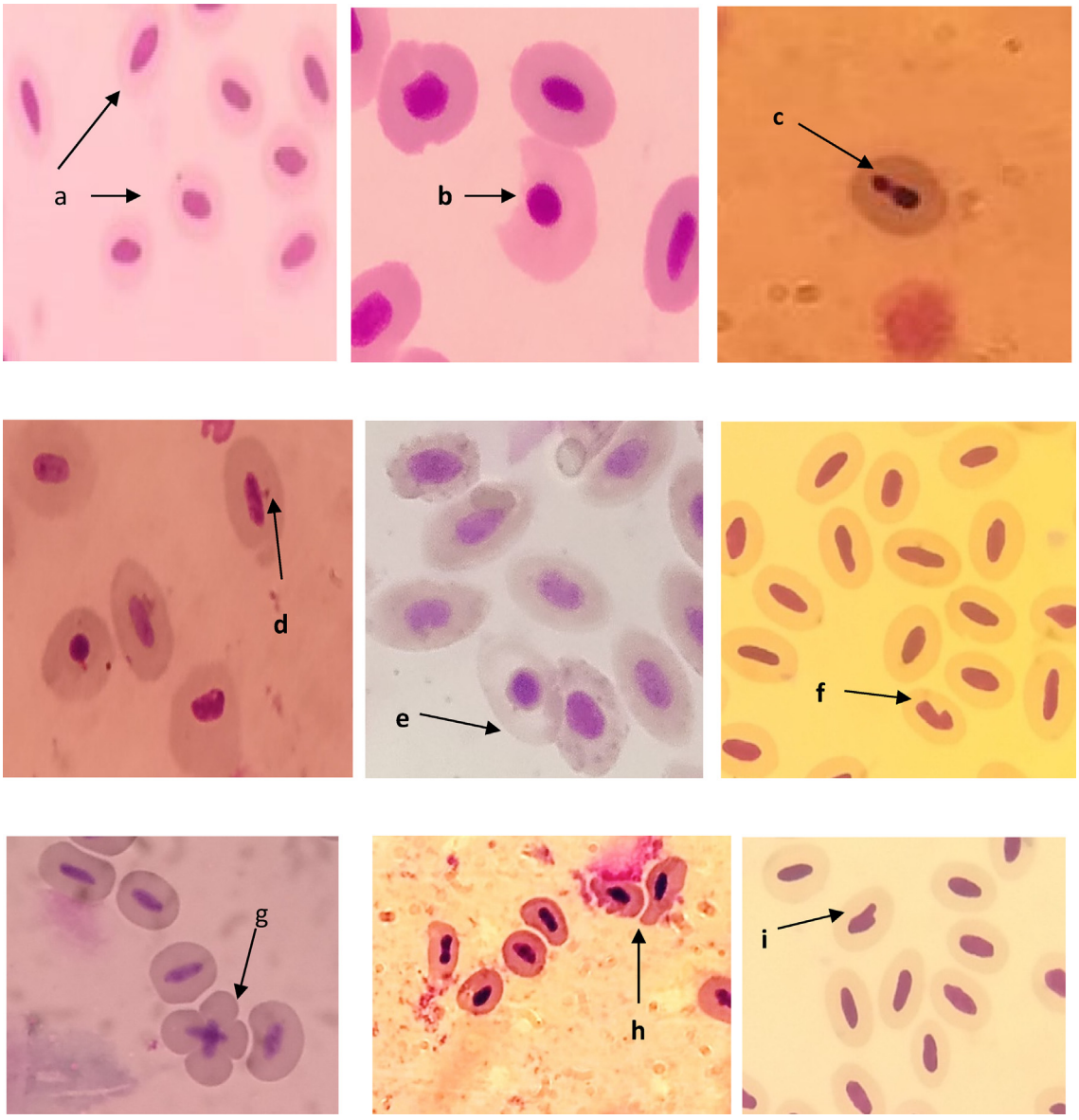

Fig. 6. Showing different cytoplasmic abnormalities in blood cells of *C. punctatus* after exposure to BPA (a) normal erythrocytes (b) lysed cell (c) binucleated cell (d) micronuclei (e) karyolised cell (f) notched nucleus (g) fused cell (h) abnormal shaped cell (i) lobed nuclei.

Besides lipid peroxidation, the activities of CAT and SOD were also assessed in the blood of *C. punctatus*. In the BPA-treated group, CAT and SOD activity decreased in the blood and the decrease was 30.51% and 54.73% with 3.81 mg/l concentration as compared to control. Similar results were reported by other studies. Decreased activities of CAT and SOD are in line with the studies of (Chitra et al., 2003). Increased lipid peroxidation in human bone mesenchymal stem cells was also revealed by (Leem et al., 2017). In another study, (Abdel-Wahab, 2014) reported that BPA induced lipid peroxidation and decreased the activity of the antioxidant defense system in rat hepatocytes. (Aboul Ezz et al., 2015) noticed BPA induced increased MDA level, depleted GSh level, and decreased CAT and AchE activities in heart of male rats. (Eid et al., 2015) found that hepatic tissue of female rat offspring exposed to BPA resulted in significantly increased MDA content while the activities of CAT, SOD, and GPx decreased significantly in the treated group. (Park and Choi, 2009) studied the effect of nonylphenol and bisphenol A on *Daphnia magna* and *Chironomus riparius* and observed increased MDA content and decreased activity of CAT in both chemical exposed groups. (Anet et al., 2019) observed increased lipid peroxidation and decreased SOD, CAT, GSH, and GST activities in *Drosophila melanogaster* treated with BPA. (Tiwari and Vanage, 2017) demonstrated bisphenol A-induced lipid peroxidation and decrease in the activity of various enzymatic and non-enzymatic antioxidants in bone marrow cells, blood lymphocytes, and testicular and epidermal tissues of male rats.

To date, it is the first report about the investigation of different antioxidant enzymes in the blood of *C. punctatus* after bisphenol A exposure. The lower values of these antioxidant enzymes might be due to increase oxidative stress and depletion of antioxidant enzymes (Ghazanfar et al., 2018; Latif et al., 2020). Our results demonstrated that BPA exposure can affect oxidative stress, including an increase in MDA levels and a decrease in CAT and SOD activity. To counteract the effects of reactive oxygen species (ROS), organisms are equipped with antioxidant defense systems that prevent cellular damage. The decreased SOD activity observed in our study may be due to the inability of the cells to generate

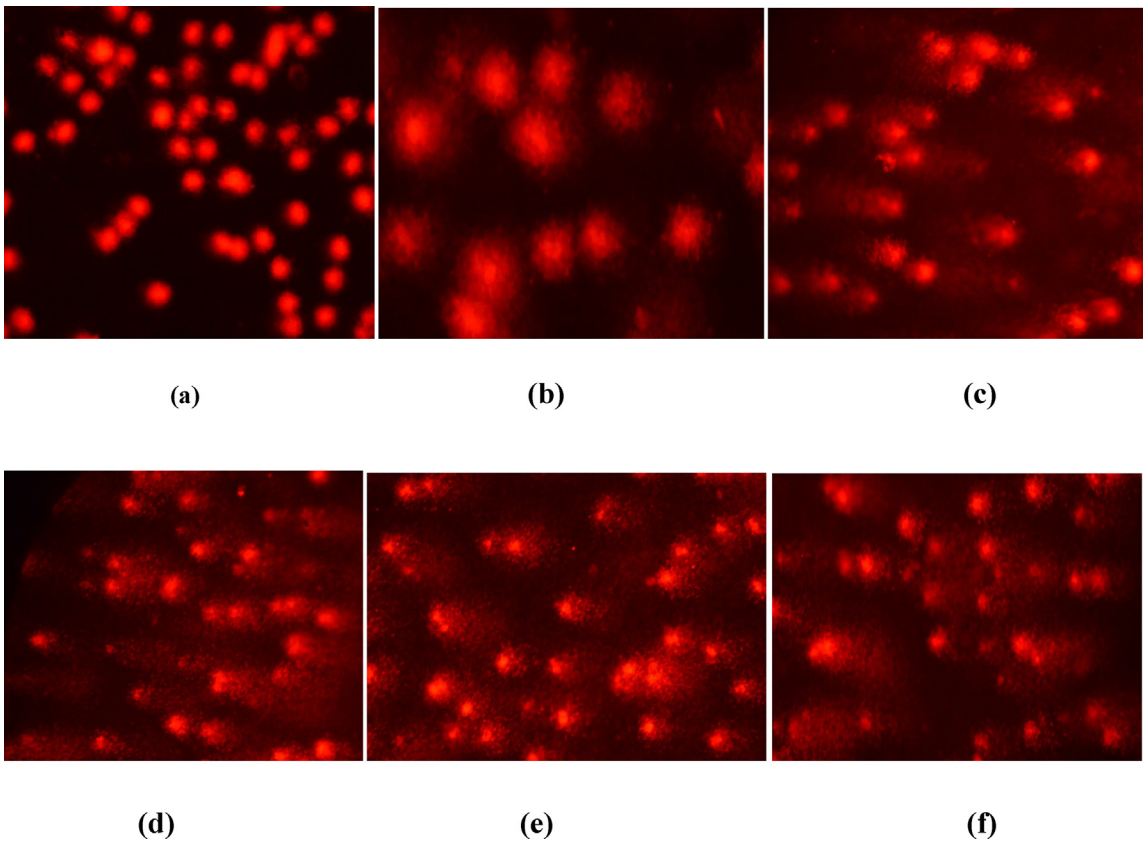

Fig. 7. Photomicrographs showing different level of DNA damage (a) control (b) mild damage (c) moderate damage (d-f) severe damage.

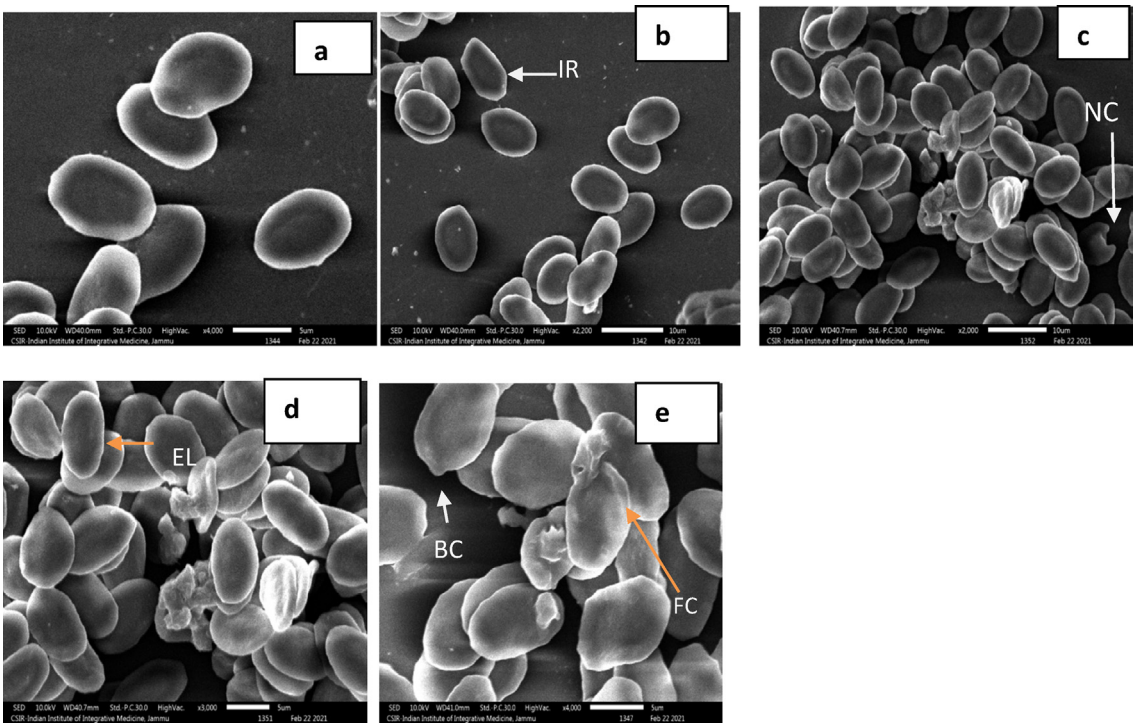

Fig. 8. Scanning electron micrographs of erythrocytes of *Channa punctatus* (a) control; (b-e) exposed to 3.81 mg/L BPA; (IR) irregular shape; (NC) notched cell; (EL) elongated cell; (BC) blebed cell; (FC) fused cells.

enough SOD, which may arise from severe cellular damage or greater functioning in combating oxidative stress.

Further, the exposure of BPA is also found to affect the DNA integrity as revealed by micronucleus and comet assays. For micronuclei assay % micronucleated cells and % aberrant cells are considered as parameters. In the present study, both duration and dose-dependent increase is observed. Significant level ($p < 0.01$) of micronucleated cell frequency and aberrant cell frequency is noticed after every exposure period. Maximum % micronucleated frequency (0.81 ± 0.01) and % aberrant cell frequency (43.89 ± 1.24) was observed for the highest concentration at 96 h of exposure. The Present study is corroborated by the work of (Yadav and Trivedi, 2009). They noticed a duration-dependent increase in micronucleus frequency in fish *C. punctatus* exposed to heavy metals. Several nuclear abnormalities (lobed nuclei, blebbed nuclei, notched nuclei, micronuclei) in erythrocytes of fish *Orechromis niloticus* (Matsumoto et al., 2006) was observed in response to heavy metals. Nuclear abnormalities like lobed nucleus, nuclear buds, elongated nucleus were may be due to the consequence of inimical effects engendered by clastogenic pollutants. All these changes occlude the chromosomal attachment due to gene amplification and leads to the emergence of notched, budded, binucleated, and other distorted nuclei (Bolognesi et al., 2006; Ergene et al., 2007).

The comet assay or the single-cell gel electrophoresis (SCGE) technique is a method for measuring DNA strand breaks in individual cells and is used to evaluate genotoxicity, DNA repair, and genome instability. The SCGE is a well-established assay for the assessment and estimation of DNA damage both *in vitro* and *in vivo*, at the individual cell level due to its capability of detecting very low-level oxidative damage induced in DNA (Bolognesi et al., 2019).

In the present study tail length (TL) and tail moment (TM) were used as parameters to assess the DNA damage. Exposed groups were found to have a significantly higher value of these parameters as compared to control showing the genotoxic effect of BPA. Previously DNA damage in spermatozoa of zebrafish (Lombó et al., 2019) and gills and lymphocytes of *C. punctatus* fish (Ali et al., 2020) due to bisphenol A has been reported.

Different researchers observed an increase in DNA damage in different fish species after exposure to various organic compounds (Rajan and Shamundeeswari. Anandan, 2017; Sharma and Chadha, 2017; Ullah et al., 2016) but the DNA damaging potential of BPA has been mainly investigated *in vitro* studies. (Ramos et al., 2019) studied the effect of Bisphenol A (BPA) on Hep-2 and MRC-5 cell lines and found that BPA induced an increase in DNA damage in the Hep-2 cell line and oxidative damage in the MRC-5 cell line. (Kose et al., 2020) observed that RWPE-1 cells exposed to BPA, bisphenol S (BPS), and bisphenol F (BPF) showed significantly higher levels of DNA damage as compared to control. (Eid et al., 2015) found a significant increase in DNA damage in the hepatic tissue of female rat offspring exposed to BPA. (Anet et al., 2019) observed significantly increased tail length and % tail intensity in cells of *Drosophila melanogaster* after treatment with BPA.

In the present study, the highest effect was seen at 24 h of exposure followed by a drop in the value but at later hours of exposure value again increases. The decrease may be due to repair of damaged DNA or replacement of highly damaged cells or both (Miyamae et al., 1997; Saleha Banu et al., 2001). Another reason may be gene activation like cytochrome p450 which activates the metabolizing enzymes and provides a defensive mechanism against genotoxicants (Wong et al., 2001). It may be due to the removal of apoptotic cells with time, which contribute to the comet in the early stages. Similar results were found by (Cavalcante et al., 2008) who tested blood and gill cells of fish and found high damage at 6 h of exposure, but at 24 h blood cells showed decreased damage and in contrast to this gill cells showed high damage at 24 h. Further (Gülsoy et al., 2015) reported the highest DNA damage at 24 h of exposure in zebrafish (*D. rerio*) treated with borax, and a decrease in values was observed at 48 h and 72 h and 96 h again the values increased. The effect of concentration was also found to be significant. The highest DNA damaging effect was observed with the highest concentration. Concentration-dependent DNA damage was found in human SK-N-MC cells treated with BDE-47 and BDE-209 (Pellacani et al., 2012). In a study, (Wang et al., 2020) exposed THP-1 cell lines to BDE-47, HBCD, and TBBPA and observed a significant increase in % tail DNA with increasing exposure concentration. (Park and Choi, 2009) studied the effect of nonylphenol and bisphenol A on *Daphnia magna* and *Chironomus riparius* and found a concentration-dependent increase in OTM in both nonylphenol and BPA exposed group. (Chen et al., 2019) found that the liver of Chinese rare minnows exposed to Tris (1,3-dichloro-2-propyl) phosphate resulted in a significant increase in DNA damage in a concentration-dependent manner.

DNA damage in our study might be due to the overproduction of free radicals related to oxidative stress. The underlying mechanism of BPA-induced genotoxicity is not very clear but it has been mainly associated with oxidative stress via ROS and lipid peroxidation (Tiwari et al., 2012) which may cause direct DNA abnormalities. DNA damage in different tissues of organisms induced by the generation of free radicals and oxidative stress has been investigated by different researchers (Ali et al., 2020; Ghaffar, 2020; Ghazanfar et al., 2018; Hussain et al., 2019)

SEM studies of erythrocytes revealed various abnormalities such as irregular shape, notched, elongated cells, cytoplasmic bleb in *C. punctatus*. Various researchers observed different abnormalities in erythrocytes of fish exposed to different xenobiotics (Dey et al., 2016; Kaur and Kaur, 2015; Mehra and Chadha, 2021). Changes in the morphology of erythrocytes of fish exposed to xenobiotics indicate the poor health status of the fish (Sawhney and Johal, 2000).

5. Conclusion

In conclusion, the acute exposure to BPA swered the haematological parameters, increased lipid peroxidation, decreased the activities of antioxidant enzymes, and caused DNA damage in blood cells of *C. punctatus*. Oxidative stress could be one of the mechanisms for the genotoxic activity of BPA as BPA-induced DNA damage was associated with elevated levels of MDA and decreased activities of CAT and SOD. Further, more study is needed to explore the molecular mechanism of BPA induced oxidative-DNA damage

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The authors are thankful to The Council of Scientific and Industrial Research CSIR New Delhi for providing financial assistance to Prince Sharma and Special Assistance Programme (SAP) of UGC for providing necessary facilities.

References

Abdel-Wahab, W.M., 2014. Thymoquinone attenuates toxicity and oxidative stress induced by bisphenol a in liver of male rats. Pakistan J. Biol. Sci. 17, 1152–1160. https://doi.org/10.3923/pjbs.2014.1152.1160.

Abdollahi, M., Ranjbar, A., Shadnia, S., Nikfar, S., Rezaie, A., 2004. Pesticides and oxidative stress: a review. Med. Sci. Monit.
Abhilash, P.C., Singh, N., 2009. Pesticide use and application: an Indian scenario. J. Hazard. Mater. https://doi.org/10.1016/j.jhazmat.2008.10.061.
Aboul Ezz, H.S., Khadrawy, Y.A., Mourad, I.M., 2015. The effect of bisphenol A on some oxidative stress parameters and acetylcholinesterase activity in the heart of male albino rats. Cytotechnology 67, 145–155. https://doi.org/10.1007/s10616-013-9672-1.
Adedeji, O.B., Adedeji, A.O., Adeyemo, O.K., Agbede, S.A., 2008. Acute toxicity of diazinon to the African catfish (Clarias gariepinus). African J. Biotechnol. 7, 651–654. https://doi.org/10.4314/ajb.v7i5.58491.
Aebi, H., 1984. [13] Catalase in Vitro. Methods Enzymol. 105, 121–126. https://doi.org/10.1016/S0076-6879(84)05016-3.
Ahuja, R., Saran, R., 1999. Alkaline single cell gel electrophoresis assay I. Protoc. Cytol. Genet. 34, 57–62.
Aiswarya, K., James, R., 2016. Effect of Bisphenol A on certain Hematological Parameters of Heteropneustes fossilis, Bloch. Int. J. Emerg. Trends Sci. Technol. 3, 4493–4497.
Ajima, M.N.O., Ogo, O.A., Akpa, L.E., Ajaero, I., 2015. Biochemical and haematological responses in African catfish Clarias gariepinus following chronic exposure to NPK (15:15:15) fertiliser. African J. Aquat. Sci. 40, 73–79. https://doi.org/10.2989/16085914.2015.1020046.
Akram, R., Iqbal, R., Hussain, R., Jabeen, F., Ali, M., 2021. Evaluation of Oxidative stress, antioxidant enzymes and genotoxic potential of bisphenol A in fresh water bighead carp (Aristichthys nobils) fish at low concentrations. Environ. Pollut. 268, 115896. https://doi.org/10.1016/j.envpol.2020.115896.
Ali, D., Falodah, F.A., Almutairi, B., Alkahtani, S., Alarifi, S., 2020. Assessment of DNA damage and oxidative stress in juvenile Channa punctatus (Bloch) after exposure to multi-walled carbon nanotubes. Environ. Toxicol. 35, 359–367. https://doi.org/10.1002/tox.22871.
Andújar, N., Gálvez-Ontiveros, Y., Zafra-Gómez, A., Rodrigo, L., Álvarez-Cubero, M.J., Aguilera, M., Monteagudo, C., Rivas, A., 2019. Bisphenol A analogues in food and their hormonal and obesogenic effects: a review. Nutrients. https://doi.org/10.3390/nu11092136.
Anet, A., Olakkaran, S., Kizhakke Purayil, A., Hunasanahally Puttaswamygowda, G., 2019. Bisphenol A induced oxidative stress mediated genotoxicity in Drosophila melanogaster. J. Hazard. Mater. 370, 42–53. https://doi.org/10.1016/j.jhazmat.2018.07.050.
Asifa, K.P., Chitra, K.C., 2015. Evaluation of Lc50 and behavioural responses of bisphenol a in the cichlid fish, and behavioural responses of bisphenol a in the cichlid fish, Etroplus Maculatus. Int. J. Curr. Res. 7, 16725–16729.
Ayala, A., Muñoz, M.F., Argüelles, S., 2014. Lipid peroxidation: Production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid. Med. Cell. Longev. https://doi.org/10.1155/2014/360438.
Banaee, M., 2013. Physiological Dysfunction in Fish After Insecticides Exposure, in: Insecticides - Development of Safer and More Effective Technologies. InTech. https://doi.org/10.5772/54742.
Benli, A., Koksal, G., 2005. The acute toxicity of ammonia on Tilapia (Oreochromis niloticus L.) larvae and fingerlings. Turkish J. Vet. Anim. Sci. 29, 339–344.
Bolognesi, C., Cirillo, S., Chipman, J.K., 2019. Comet assay in ecogenotoxicology: applications in Mytilus sp. Mutat. Res. - Genet. Toxicol. Environ. Mutagen. 842, 50–59. https://doi.org/10.1016/j.mrgentox.2019.05.004.
Bolognesi, C., Perrone, E., Roggieri, P., Pampanin, D.M., Sciutto, A., 2006. Assessment of micronuclei induction in peripheral erythrocytes of fish exposed to xenobiotics under controlled conditions. Aquat. Toxicol. 78, S93–S98. https://doi.org/10.1016/j.aquatox.2006.02.015.
Bonds, R.S., Midoro-Horiuti, T., 2013. Estrogen effects in allergy and asthma. Curr. Opin. Allergy Clin. Immunol. 13 (1), 92–99.
Bonefeld-Jørgensen, E.C., Long, M., Hofmeister, M.V., Vinggaard, A.M., 2007. Endocrine-disrupting potential of bisphenol A, bisphenol A dimethacrylate, 4-n-nonylphenol, and 4-n-octylphenol in vitro: new data and a brief review. Environmental health perspectives 115 (Suppl 1), 69–76.
Bowes, D.A., Halden, R.U., 2019. Breast Cancer and Dietary Intake of Endocrine Disruptors: a Review of Recent Literature. Curr. Pathobiol. Rep. https://doi.org/10.1007/s40139-019-00199-1
Buha Djordjevic, A., Antonijevic, E., Curcic, M., Milovanovic, V., Antonijevic, B., 2020. Endocrine-disrupting mechanisms of polychlorinated biphenyls. Curr. Opin. Toxicol. https://doi.org/10.1016/j.cotox.2019.10.006.
Cano-Nicolau, J., Vaillant, C., Pellegrini, E., Charlier, T.D., Kah, O., Coumailleau, P., 2016. Estrogenic effects of several BPA analogs in the developing zebrafish brain. Front. Neurosci. 10, 112. https://doi.org/10.3389/fnins.2016.00112.
Cargnelutti, F., Di, A., Francesco, N., Iva, P., Matteo, S., Grazia, M., Donatella, T., Carlo, P., 2020. Effects of endocrine disruptors on fetal testis development, male puberty, and transition age. Endocrine. https://doi.org/10.1007/s12020-020-02436-9.
Cariati, F., Carbone, L., Conforti, A., Bagnulo, F., Peluso, S.R., Carotenuto, C., Buonfantino, C., Alviggi, E., Alviggi, C., Strina, I., 2020. Bisphenol A-Induced epigenetic changes and its effects on the male reproductive system. Front. Endocrinol. 11, 1–11.
Cavalcante, D.G.S.M., Martinez, C.B.R., Sofia, S.H., 2008. Genotoxic effects of Roundup® on the fish Prochilodus lineatus. Mutat. Res. - Genet. Toxicol. Environ. Mutagen. 655, 41–46. https://doi.org/10.1016/j.mrgentox.2008.06.010.
Chen, R., Hou, R., Hong, X., Yan, S., Zha, J., 2019. Organophosphate fl ame retardants (OPFRs) induce genotoxicity in vivo : A survey on apoptosis, DNA methylation, DNA oxidative damage, liver metabolites, and transcriptomics. Environ. Int. 130,. https://doi.org/10.1016/j.envint.2019.104914 104914.
Chen, W., Lau, S.W., Fan, Y., Wu, R.S.S., Ge, W., 2017. Juvenile exposure to bisphenol A promotes ovarian differentiation but suppresses its growth – Potential involvement of pituitary follicle-stimulating hormone. Aquat. Toxicol. 193, 111–121. https://doi.org/10.1016/j.aquatox.2017.10.008.
Chitra, K.C., Latchoumycandane, C., Mathur, P.P., 2003. Induction of oxidative stress by bisphenol A in the epididymal sperm of rats. Toxicology 185, 119–127. https://doi.org/10.1016/S0300-483X(02)00597-8.
Darbre, P.D., 2020. Chemical components of plastics as endocrine disruptors: Overview and commentary. Birth Defects Res. 112, 1300–1307. https://doi.org/10.1002/bdr2.1778.
Delfosse, V., Grimaldi, M., Cavaillès, V., Balaguer, P., Bourguet, W., 2014. Structural and Functional Profiling of Environmental Ligands for Estrogen Receptors. Environ Health Perspect. 122 (12), 1306–1313.
Dey, S., Choudhury, M.D., Das, S., 2016. Sublethal effects of pulp and paper mill effluent on two commonly cultured carps: a SEM- and EDS-based hematological biomarker analysis. Fish Physiol. Biochem. 42, 1791–1805. https://doi.org/10.1007/s10695-016-0258-7.
Donohue, K.M., Miller, R.L., Perzanowski, M.S., Just, A.C., Hoepner, L.A., Arunajadai, S., Canfield, S., Resnick, D., Calafat, A.M., Perera, F.P., Whyatt, R.M., 2013. Prenatal and postnatal bisphenol A exposure and asthma development among inner-city children. J Allergy Clin Immunol. 131 (3), 736–742.
Eid, J.I., Eissa, S.M., El-Ghor, A.A., 2015. Bisphenol A induces oxidative stress and DNA damage in hepatic tissue of female rat offspring. J. Basic Appl. Zool. 71, 10–19. https://doi.org/10.1016/j.jobaz.2015.01.006.
Ergene, S., Çavaş, T., Çelik, A., Köleli, N., Kaya, F., Karahan, A., 2007. Monitoring of nuclear abnormalities in peripheral erythrocytes of three fish species from the Goksu Delta (Turkey): Genotoxic damage in relation to water pollution. Ecotoxicology 16, 385–391. https://doi.org/10.1007/s10646-007-0142-4.
Faheem, M., Khaliq, S., Ahmad, H.U., Lone, K.P., 2017. Bisphenol-A (BPA) alters plasma thyroid hormones and sex steroids in female Pakistani major carp (Catla catla; Cyprinidae). Pak. Vet. J. 37, 326–330.
Fenichel, P., Chevalier, N., Brucker-Davis, F., 2013. Bisphenol A: An endocrine and metabolic disruptor. *Ann Endocrinol* (Paris). 74 (3), 211–220.
Finney, D., 1971. Probit Analysis. Cambridge University Press, Cambridge UK.
Franklin, J.L., 2011. Redox regulation of the intrinsic pathway in neuronal apoptosis. Antioxidants Redox Signal. https://doi.org/10.1089/ars.2010.3596.
Gascon, M., Casas, M., Morales, E., Valvi, D., Ballesteros-Gómez, A., Luque, N., Rubio, S., Monfort, N., Ventura, R., Martínez, D., Sunyer, J., 2015. Prenatal exposure to bisphenol A and phthalates and childhood respiratory tract infections and allergy. J Allergy Clin Immunol. 135 (2), 370–378.
Ghaffar, A., 2020. Dose and Time-Related Pathological and Genotoxic Studies on Thiamethoxam in Fresh Water Fish (Labeo rohita) in Pakistan. Pak. Vet. J. 40, 151–156 https://doi.org/10.29261/pakvetj/2020.002.
Ghaffar, A., Hussain, R., Abbas, G., Kalim, M., Khan, A., Ferrando, S., Gallus, L., Ahmed, Z., 2018. Fipronil (Phenylpyrazole) induces hemato-biochemical, histological and genetic damage at low doses in common carp, Cyprinus carpio (Linnaeus, 1758). Ecotoxicology 27, 1261–1271. https://doi.org/10.1007/s10646-018-1979-4.
Ghazanfar, M., Shahid, S., Qureshi, I.Z., 2018. Vitamin C attenuates biochemical and genotoxic damage in common carp (Cyprinus carpio) upon joint exposure to combined toxic doses of fipronil and buprofezin insecticides. Aquat. Toxicol. 196, 43–52. https://doi.org/10.1016/j.aquatox.2017.12.015.
Gülsoy, N., Yavaş, C., Mutlu, Ö., 2015. Genotoxic effects of boric acid and borax in zebrafish, danio rerio using alkaline comet assay. EXCLI J. 14, 890–899 https://doi.org/10.17179/excli2015-404.
Hussain, R., Ali, F., Javed, M.T., Jabeen, G., Ghaffar, A., Khan, I., Liaqat, S., Hussain, T., Abbas, R.Z., Riaz, A., Gul, S.T., Ghori, M.T., 2019. Clinico-hematological, serum biochemical, genotoxic and histopathological effects of trichlorfon in adult cockerels. Toxin Rev. https://doi.org/10.1080/15569543.2019.1673422.
Jain, N.C. (1986). Schalm's Veterinary Haematology 4th edition. Lea and Febiger, Philadelphia pp 1221. [WWW Document], n.d. URL http://www.sciepub.com/reference/288993 (accessed 2.10.21).
Jalal, N., Surendranath, A.R., Pathak, J.L., Yu, S., Chung, C.Y., 2018. Bisphenol A (BPA) the mighty and the mutagenic. Toxicol. Reports. https://doi.org/10.1016/j.toxrep.2017.12.013
Krishnapriya, Kaliappan, Shobana, Ganesan, Narmadha, Subramaniam, Ramesh, Mathan, Maruthappan, Vettaegounder, 2017. Sublethal concentration of bisphenol A induces hematological and biochemical responses in an Indian major carp Labeo rohita. Ecohydrol. Hydrobiol. 17, 306–313. https://doi.org/10.1016/j.ecohyd.2017.06.003.
Kaur, K., Kaur, A., 2015. Fish Erythrocytes as Biomarkers for the Toxicity of Sublethal Doses of an Azo Dye, Basic Violet-1 (CI: 42535). Microsc. Microanal. 21, 264–273. https://doi.org/10.1017/S1431927614013609.
Kono, Y., 1978. Generation of superoxide radical during autoxidation of hydroxylamine and an assay for superoxide dismutase. Arch. Biochem. Biophys. 186, 189–195. https://doi.org/10.1016/0003-9861(78)90479-4.
Kose, O., Rachidi, W., Beal, D., Fayyad-kazan, H., Gumusel, B.K., 2020. The effects of different bisphenol derivatives on oxidative stress , DNA damage and DNA repair in RWPE-1 cells : A comparative study 643–654. https://doi.org/10.1002/jat.3934
KUmar, M., Kumar, P., Devi, S., 2015. Toxicity of Copper Sulphate on Behavioural Parameter and Respiratory Surveillance in Freshwater Catfish, Clarias Batrachus (Linn.) 3, 22–28.
Kumar, R., Nagpure, N.S., Kushwaha, B., Srivastava, S.K., Lakra, W.S., 2010. Investigation of the genotoxicity of malathion to freshwater teleost fish channa punctatus (Bloch) using the micronucleus test and comet assay. Arch.

Environ. Contam. Toxicol. 58, 123–130. https://doi.org/10.1007/s00244-009-9354-3.
Latif, M., Faheem, M., Asmatullah, 2020. Study of oxidative stress and histo-biochemical biomarkers of diethyl phthalate induced toxicity in a cultureable fish, Labeo rohita. Pak. Vet. J. 40, 202–208. https://doi.org/10.29261/pakvetj/2019.108
Leem, Y.H., Oh, S., Kang, H.J., Kim, J.H., Yoon, J., Chang, J.S., 2017. BPA-toxicity via superoxide anion overload and a deficit in β-catenin signaling in human bone mesenchymal stem cells. Environ. Toxicol. 32, 344–352. https://doi.org/10.1002/tox.22239.
Li, D., Bi, R., Chen, H., Mu, L., Zhang, L., Chen, Q., Xie, H., Luo, Y., Xie, L., 2017. The acute toxicity of bisphenol A and lignin-derived bisphenol in algae, daphnids, and Japanese medaka. Environ. Sci. Pollut. Res. 24, 23872–23879. https://doi.org/10.1007/s11356-017-0018-y.
Li, D.K., Zhou, Z., Miao, M., He, Y., Wang, J., Ferber, J., Herrinton, L.J., Gao, E., Yuan, W., 2011. Urine bisphenol-A (BPA) level in relation to semen quality. Fertil Steril. 95 (2), 625–630.
Lombó, M., Getino-álvarez, L., Depincé, A., Labbé, C., Herráez, M.P., 2019. Embryonic exposure to bisphenol a impairs primordial germ cell migration without jeopardizing male breeding capacity. Biomolecules 9. https://doi.org/10.3390/biom9080307.
Matsumoto, S.T., Mantovani, M.S., Malaguttii, M.I.A., Dias, A.L., Fonseca, I.C., Marin-Morales, M.A., 2006. Genotoxicity and mutagenicity of water contaminated with tannery effluents as evaluated by the micronucleus test and comet assay using the fish Oreochromis niloticus and chromosome aberrations in onion root-tips. Genet. Mol. Biol. 29, 148–158. https://doi.org/10.1590/S1415-47572006000100028.
Mckenzie, D.J., Shingles, A., Claireaux, G., Domenici, P., 2009. Sublethal concentrations of ammonia impair performance of the teleost fast-start escape response. Physiol. Biochem. Zool. 82, 353–362. https://doi.org/10.1086/590218.
Mehra, S., Chadha, P., 2021. Naphthalene-2-sulfonate induced toxicity in blood cells of freshwater fish Channa punctatus using comet assay, micronucleus assay and ATIR-FTIR approach. Chemosphere 265. https://doi.org/10.1016/j.chemosphere.2020.129147.
Michałowicz, J., 2014. Bisphenol A - Sources, toxicity and biotransformation. Environ. Toxicol. Pharmacol. https://doi.org/10.1016/j.etap.2014.02.003
Mishra, A.K., Mohanty, B., 2008. Acute toxicity impacts of hexavalent chromium on behavior and histopathology of gill, kidney and liver of the freshwater fish, Channa punctatus (Bloch). Environ. Toxicol. Pharmacol. 26, 136–141. https://doi.org/10.1016/j.etap.2008.02.010.
Miyamae, Y., Iwasaki, K., Kinae, N., Tsuda, S., Murakami, M., Tanaka, M., Sasaki, Y.F., 1997. Detection of DNA lesions induced by chemical mutagens using the single-cell gel electrophoresis (Comet) assay. 2. Relationship between DNA migration and alkaline condition. Mutat. Res. - Genet. Toxicol. Environ. Mutagen. 393, 107–113. https://doi.org/10.1016/S1383-5718(97)00091-0.
Molina, A., Abril, N., Morales-Prieto, N., Monterde, J., Ayala, N., Lora, A., Moyano, R., 2018. Hypothalamic-pituitary-ovarian axis perturbation in the basis of bisphenol A (BPA) reproductive toxicity in female zebrafish (Danio rerio). Ecotoxicol. Environ. Saf. 156, 116–124. https://doi.org/10.1016/j.ecoenv.2018.03.029.
Monteiro, S.M., Fontainhas-Fernandes, A., Sousa, M., 2010. An immunohistochemical study of gill epithelium cells in the Nile tilapia. Oreochromis niloticus. Folia Histochem. Cytobiol. 48, 112–121. https://doi.org/10.2478/v10042-008-0105-5.
Nagelkerken, I., Munday, P.L., 2016. Animal behaviour shapes the ecological effects of ocean acidification and warming: Moving from individual to community-level responses. Glob. Chang. Biol. 22, 974–989. https://doi.org/10.1111/gcb.13167.
Naz, S., Hussain, R., Ullah, Q., Chatha, A.M.M., Shaheen, A., Khan, R.U., 2020. Toxic effect of some heavy metals on hematology and histopathology of major carp (Catla catla). Environ. Sci. Pollut. Res. https://doi.org/10.1007/s11356-020-10980-0
Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95, 351–358. https://doi.org/10.1016/0003-2697(79)90738-3.
Pal, S., Reddy, P.B., 2018. BISPHENOL A (BPA) INDUCEDHISTOPATHOLOGICAL AND BIOCHEMICAL ALTERATIONS IN THE LIVER AND KIDNEY OF STINGING CAT FISH HETEROPNEUSTES FOSSILIS. Trends Fish. Res. 7, 67–74.
Park, S.Y., Choi, J., 2009. Genotoxic effects of nonylphenol and bisphenol a exposure in aquatic biomonitoring species: Freshwater crustacean, daphnia magna, and aquatic midge, chironomus riparius. Bull. Environ. Contam. Toxicol. 83, 463–468. https://doi.org/10.1007/s00128-009-9745-1.
Parveen, S., Singh, D., Bharose, R., Rout, S., Khan, M.A., Ansari, E.F., 2017. Tannery effluent effect on the haematological parameters of freshwater fish, Channa punctatus. J. Appl. Nat. Sci. 9, 201–205 https://doi.org/10.31018/jans.v9i1.1173.
Pellacani, C., Buschini, A., Galati, S., Mussi, F., Franzoni, S., Costa, L.G., 2012. Evaluation of DNA damage induced by 2 polybrominated diphenyl ether flame retardants (BDE-47 and BDE-209) in SK-N-MC cells. Int. J. Toxicol. 31, 372–379. https://doi.org/10.1177/1091581812449663.
Porreca, I., Ulloa Severino, L., D'Angelo, F., Cuomo, D., Ceccarelli, M., Altucci, L., Amendola, E., Nebbioso, A., Mallardo, M., De Felice, M., Ambrosino, C., 2016. "Stockpile" of slight transcriptomic changes determines the indirect genotoxicity of low-dose BPA in thyroid cells. PLoS One 11. https://doi.org/10.1371/journal.pone.0151618.
Rajan, A.P., Shamundeeswari.Anandan, 2017. INVESTIGATION OF CARCINOGENIC AND MUTAGENIC PROPERTY OF FOOD COLOR USING CAT FISHCLARIAS BATRACHUSBY USING ALKALINE SINGLE-CELL GEL ELECTROPHORESIS (COMET) ASSAY AND MICRONUCLEUS ASSAY. Int. J. Pharmacol. Clin. Sci. 4, 29–34. https://doi.org/10.5281/ZENODO.831536
Ramos, C., Ladeira, C., Zeferino, S., Dias, A., Faria, I., Cristovam, E., Gomes, M., Ribeiro, E., 2019. Cytotoxic and genotoxic effects of environmental relevant concentrations of bisphenol A and interactions with doxorubicin. Mutat. Res. - Genet. Toxicol. Environ. Mutagen. 838, 28–36. https://doi.org/10.1016/j.mrgentox.2018.11.009.
Rao, J.V., 2006. Toxic effects of novel organophosphorus insecticide (RPR-V) on certain biochemical parameters of euryhaline fish, Oreochromis mossambicus. Pestic. Biochem. Physiol. 86, 78–84. https://doi.org/10.1016/j.pestbp.2006.01.008.
Saili, K.S., Corvi, M.M., Weber, D.N., Patel, A.U., Das, S.R., Przybyla, J., Anderson, K.A., Tanguay, R.L., 2012. Neurodevelopmental low-dose bisphenol A exposure leads to early life-stage hyperactivity and learning deficits in adult zebrafish. Toxicology 291, 83–92. https://doi.org/10.1016/j.tox.2011.11.001.
Saleha Banu, B., Danadevi, K., Rahman, M.F., Ahuja, Y.R., Kaiser, J., 2001. Genotoxic effect of monocrotophos to sentinel species using comet assay. Food Chem. Toxicol. 39, 361–366. https://doi.org/10.1016/S0278-6915(00)00141-1.
Sawhney, A.K., Johal, M.S., 2000. Erythrocyte Alterations Induced by Malathion in Channa punctatus (Bloch). Bull. Environ. Contam. Toxicol. 64, 398–405. https://doi.org/10.1007/s001280000014.
Scarano, W.R., Bedrat, A., Alonso-Costa, L.G., Aquino, A.M., Fantinatti, B.E.A., Justulin, L.A., Barbisan, L.F., Freire, P.P., Flaws, J.A., Lemos, B., 2019. Exposure to an Environmentally Relevant Phthalate Mixture during Prostate Development Induces MicroRNA Upregulation and Transcriptome Modulation in Rats. Toxicol. Sci. 171, 84–97. https://doi.org/10.1093/toxsci/kfz141.
Shafei, A., Matbouly, M., Mostafa, E., Al Sannat, S., Abdelrahman, M., Lewis, B., Muhammad, B., Mohamed, S., Mostafa, R.M., 2018a. Stop eating plastic, molecular signaling of bisphenol A in breast cancer. Environ. Sci. Pollut. Res. https://doi.org/10.1007/s11356-018-2540-y
Shafei, A., Ramzy, M.M., Hegazy, A.I., Husseny, A.K., EL-hadary, U.G., Taha, M.M., Mosa, A.A., 2018b. The molecular mechanisms of action of the endocrine disrupting chemical bisphenol A in the development of cancer. Gene. https://doi.org/10.1016/j.gene.2018.01.016
Sharma, M., Chadha, P., 2017. Widely used non-ionic surfactant 4-nonylphenol: showing genotoxic effects in various tissues of Channa punctatus. Environ. Sci. Pollut. Res. 24, 11331–11339. https://doi.org/10.1007/s11356-017-8759-1.
Sharma, M., Chadha, P., 2015. Acute Toxicity of 4-nonylphenol on Haemotological profile of Fresh water Fish Channa punctatus. Research Journal of Recent Sciences.
Sharma, M., Thakur, J., Verma, S., Sharma, P., 2019. Behavioural responses in effect to chemical stress in fish: A review. Int. J. Fish. Aquat. Stud. 7, 01–05.
Thornton, J., Lewis, J., Lebrun, C.M., Licskai, C.J., 2012. Clinical characteristics of women with menstrual-linked asthma. Respir Med. 106 (9), 1236–1243.
Tiwari, D., Kamble, J., Chilgunde, S., Patil, P., Maru, G., Kawle, D., Bhartiya, U., Joseph, L., Vanage, G., 2012. Clastogenic and mutagenic effects of bisphenol A: An endocrine disruptor. Mutat. Res. - Genet. Toxicol. Environ. Mutagen. 743, 83–90. https://doi.org/10.1016/j.mrgentox.2011.12.023.
Tiwari, D., Vanage, G., 2017. Bisphenol A Induces Oxidative Stress in Bone Marrow Cells, Lymphocytes, and Reproductive Organs of Holtzman Rats. Int. J. Toxicol. 36, 142–152. https://doi.org/10.1177/1091581817691224.
TSAI, W.-T., 2006. Human Health Risk on Environmental Exposure to Bisphenol-A: A Review. J. Environ. Sci. Heal. Part C 24, 225–255. https://doi.org/10.1080/10590500600936482
Ullah, S., Begum, M., Dhama, K., Ahmad, S., Hassan, S., Alam, I., 2016. Malathion induced DNA damage in freshwater fish, Labeo rohita (Hamilton, 1822) using alkaline single cell gel electrophoresis. Asian J. Anim. Vet. Adv. 11, 98–105. https://doi.org/10.3923/ajava.2016.98.105.
Vieira, W.T., de Farias, M.B., Spaolonzi, M.P., da Silva, M.G.C., Vieira, M.G.A., 2020. Removal of endocrine disruptors in waters by adsorption, membrane filtration and biodegradation. A review. Environ. Chem. Lett. https://doi.org/10.1007/s10311-020-01000-1
Wang, B., Wang, H., Han, D., Chen, J., Yin, Y., 2020. Studying the mixture effects of brominated flame retardants and metal ions by comet assay. Environ. Pollut. 267,. https://doi.org/10.1016/j.envpol.2020.115677 115677.
Wang, Q., Yang, H., Yang, M., Yu, Y., Yan, M., Zhou, L., Liu, X., Xiao, S., Yang, Y., Wang, Y., Zheng, L., Zhao, H.H., Li, Y., 2019. Toxic effects of bisphenol A on goldfish gonad development and the possible pathway of BPA disturbance in female and male fish reproduction. Chemosphere 221, 235–245. https://doi.org/10.1016/j.chemosphere.2019.01.033.
Wang, X., Dong, Q., Chen, Y., Jiang, H., Xiao, Q., Wang, Y., Li, W., Bai, C., Huang, C., Yang, D., 2013. Bisphenol A affects axonal growth, musculature and motor behavior in developing zebrafish. Aquat. Toxicol. 142–143, 104–113. https://doi.org/10.1016/j.aquatox.2013.07.011.
Watkins, D.J., Sánchez, B.N., Téllez-Rojo, M.M., Lee, J.M., Mercado-García, A., Blank-Goldenberg, C., Peterson, K.E., Meeker, J.D., 2017. Phthalate and bisphenol A exposure during in utero windows of susceptibility in relation to reproductive hormones and pubertal development in girls. Environ. Res. 159, 143–151. https://doi.org/10.1016/j.envres.2017.07.051.
Wong, B.B.M., Candolin, U., 2015. Behavioral responses to changing environments. Behav. Ecol. https://doi.org/10.1093/beheco/aru183
Wong, C.K.C., Yeung, H.Y., Woo, P.S., Wong, M.H., 2001. Specific expression of cytochrome P4501A1 gene in gill, intestine and liver of tilapia exposed to

coastal sediments. Aquat. Toxicol. 54, 69–80. https://doi.org/10.1016/S0166-445X(00)00173-9.

Xing, X., Li, M., Yuan, L., Song, M., Ren, Q., Shi, G., Meng, F., Wang, R., 2016. The protective effects of taurine on acute ammonia toxicity in grass carp Ctenopharynodon idellus. Fish Shellfish Immunol. 56, 517–522. https://doi.org/10.1016/j.fsi.2016.08.005.

Xu, J., Zhou, L., Wang, S., Zhu, J., Liu, T., Jia, Y., Sun, D., Chen, H., Wang, Q., Xu, F., Zhang, Y., Liu, H., Zhang, T., Ye, L., 2018. Di-(2-ethylhexyl)-phthalate induces glucose metabolic disorder in adolescent rats. Environ. Sci. Pollut. Res. 25, 3596–3607. https://doi.org/10.1007/s11356-017-0738-z.

Yadav, K.K., Trivedi, S.P., 2009. Sublethal exposure of heavy metals induces micronuclei in fish, Channa punctata. Chemosphere 77, 1495–1500. https://doi.org/10.1016/j.chemosphere.2009.10.022.

Yang, Yunjia, Yang, Yi, Zhang, J., Shao, B., Yin, J., 2019. Assessment of bisphenol A alternatives in paper products from the Chinese market and their dermal exposure in the general population. Environ. Pollut. 244, 238–246. https://doi.org/10.1016/j.envpol.2018.10.049.

Zhou, A., Chang, H., Huo, W., Zhang, B., Hu, J., Xia, W., Chen, Z., Xiong, C., Zhang, Y., Wang, Y., Xu, S., 2017. Prenatal exposure to bisphenol A and risk of allergic diseases in early life. Pediatr. Res. 81 (6), 851–856.

Zhou, L., Chen, H., Xu, Q., Han, X., Zhao, Y., Song, X., Zhao, T., Ye, L., 2019. The effect of di-2-ethylhexyl phthalate on inflammation and lipid metabolic disorder in rats. Ecotoxicol. Environ. Saf. 170, 391–398. https://doi.org/10.1016/j.ecoenv.2018.12.009.