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Micronuclei and other nuclear abnormalities in phorate exposed fish, *Channa punctatus*

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Abstract

Aim: The present study aims to establish morphology-based nuclear abnormalities (NAs) including micronuclei (MN) as effective and sensitive genotoxic endpoint biomarkers in fish against the sub-lethal exposure of phorate.

Methodology: Fish, *Channa punctatus* (35 ± 3.0 g; 14.5 ± 1.0 cm) were randomly exposed in two sets, treated group 1 and 2 (0.0375 mg l⁻¹ and 0.075 mg l⁻¹ of phorate, respectively) along with a simultaneous control (0 mg l⁻¹). The blood was sampled after 30 days.

Results: A significant (p < 0.05) induction in reactive oxygen species (ROS) coupled with elevated frequency of blood cells showing micronuclei along with the gross appearance of notched nuclei, curved nuclei, blebbed nuclei, kidney-shaped nuclei, V-shaped nuclei, nuclear buds, nucleoplasmic bridges, dumbbell nuclei, and condensed/rounded nuclei were recorded in a dose-dependent manner.

Interpretation: The findings of present investigation can, thus, be reasonably employed as sensitive cytotoxic endpoint biomarkers as early warning tools to evaluate the genotoxicity induced by phorate in aquatic organisms, like fish.

Key words: *Channa punctatus*, Micronuclei, Molecular biomarkers, Nuclear abnormalities, Oxidative stress, Phorate

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Introduction

Organophosphate pesticides (Ops), as crop protection chemicals, were introduced in India in the 1960s (Aktar *et al.*, 2009). The nation consumes 76% of pesticides as insecticides, while the worldwide rate of their consumption is about 44% (Bhardwaj and Sharma, 2013). Further, in Asia, India ranks first in pesticide production and holds twelfth position in the world (Abhilash and Singh, 2009). Phorate, being a broad spectrum OP, is widely used against different pests (Myers *et al.*, 2016). The WHO has classified phorate as one of the most hazardous pesticides of class Ia in 2009. Phorate though has been banned or restricted in use in many countries, it is still used in India. This generates ecological constraints, particularly in non-target organisms of aquatic habitat (Mostafalou and Abdollahi, 2013).

During biotransformation, phorate primarily gets converted into an oxon, by cytochrome P450, which inhibits the activity of acetylcholinesterase (AChE) (Hodgson, 2012). The inhibition of AChE promotes ROS (reactive oxygen species) build-up and consequently enhances lipid per-oxidation and, thus, the oxidative stress, as evident in fish exposed to other crop protection chemicals, deltamethrin and cypermethrin (Slaninova *et al.*, 2009). In fact, over production of ROS generates superoxide anions (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl ions ($OH^\cdot$) (Saqib *et al.*, 2012). Normally, these ROS are involved in cytoplasmic homeostasis by maintaining the cellular signaling pathways, however, their excess production contributes to oxidative stress leading to molecular damages via retrograde redox signaling among the cell organelles, *i.e.*, mitochondria to cytosol and nucleus (Murphy, 2009). Further, extended subsistence of oxidative stress leads to genetic instability due to unresolved DNA repair complexes (Iarmarcovai *et al.*, 2008). These instabilities in fish can be easily estimated by various nuclear anomalies/abnormalities that offer concrete information for DNA damage and genomic or chromatin instability triggered by xenobiotics. Earlier studies have also reported generation of aforementioned nuclear anomalies in fishes exposed to crop protection chemicals other than phorate (Ahmad and Ahmad, 2016). These nuclear anomalies can be adequately used for instant diagnosis of genotoxic damage in human beings too (Kirsch-Volders and Fenech, 2001).

Nuclear anomalies are mostly formed due to condensation of genetic material inside the nucleus and under the influence of mitochondrial factors. Among these, apoptosis inducing factor is the major one which is responsible for such condensation (Candé *et al.*, 2002). Among nuclear abnormalities, micronuclei, a chromatinic body, is an outcome of the DNA strand breaks and/or formed by the condensation of chromatin fragments. Micronuclei assay is a rapid and extensively used technique to study the non-specific genomic damage (Trivedi *et al.*, 2020). Moreover, micronuclei induction also indicates misrepair of DNA strand breaks (Klobučar *et al.*, 2010). An

elevation in micronuclei frequency was reported in fish exposed to a pesticide, fenvalerate (Chaudhari and Saxena, 2015) and also in blood cells of humans after their exposure to an herbicide 2, 4-dichlorophenoxyacetic acid (Holland *et al.*, 2002).

Nuclear abnormalities promptly illustrate the induction of genotoxic damages in blood cells, especially erythrocytes of animals exposed to xenobiotics. They can be efficiently used for the diagnosis of chromosomal modifications (Thomas *et al.*, 2003). These nuclear abnormalities may be considered better biomarkers than cytotoxic indicators due to their higher sensitivity and more perceptibility (Ahmad and Ahmad, 2016). In fact, to understand the eco-genotoxicological manifestations, nuclear abnormalities may be considered as genotoxic endpoint biomarkers. Further, the correlation approaches among aforesaid nuclear abnormalities and oxidative stress are adequately able to decipher the strength of relationships among them. Thus, the present study reflects ample prospects for less expensive and rapid screening methodologies for assessment of genotoxicological perturbations in fish for routine biomonitoring of contaminated aquatic habitats.

Materials and Methods

Fish collection and their acclimatization : For present study, live and active fishes (35 ± 3.0 g; 14.5 ± 1.0 cm), *C. punctatus*, were hand netted with the help of local fishermen from suburban aquatic habitats of Lucknow, India. In the laboratory, initially, fishes were dipped in 0.05% solution of potassium permanganate ($KMnO_4$) for 4 min to get rid of epidermal contagions. Fish were subjected for acclimatization. Food pellets (Perfect Companion Group Company Limited, Thailand) at a rate of 2% of body weight were provided to fishes, daily. Feeding was discontinued for one day before *in-vivo* experiment.

Experimental design : For *in-vivo* study, 30 specimens were randomly distributed into three groups: control (0 mg l^{-1}), T1 (0.0375 mg l^{-1}) and T2 (0.075 mg l^{-1}), each containing 10 specimens. The dose of phorate selected for T1 (0.0375 mg l^{-1}) and T2 (0.075 mg l^{-1}) were 20 and 10 times lower to reported LC_{50} value 0.75 mg l^{-1} for *C. punctatus* (Ratn *et al.*, 2017). Experiments were run in triplicate for 30 day and fish were fed only with maintenance feed at 7 day intervals. Every day, food remains and excretory wastes were removed from each aquaria. After 30 days, fish were anaesthetized with 0.01% (v/w) diethyl ether. Blood sampling was done in ethylenediaminetetraacetic acid (EDTA; 1.8 mg ml^{-1}) coated vials through heart puncture by using a heparinized syringe for assessment of ROS and associated nuclear abnormalities.

Estimation of ROS and CFS : The phorate-induced ROS activity in erythrocytes of *C. punctatus* and subsequent fluorescence were estimated by employing 2', 7'-dichlorodihydrofluorescein diacetate ($20 \mu\text{M}$, DCFH-DA; Sigma Aldrich, USA) dye and a

fluorescent microscope, respectively, by following the modified method (Ratn *et al.*, 2018). Two thousand cells were counted to quantify the percentage of erythrocytes showing ROS-induced fluorescent signals (CFS).

Evaluation of nuclear abnormalities : Phorate induced chromosomal breakage or loss of whole chromosome in terms of micronuclei induction, and aforesaid nuclear abnormalities were assessed in fish of each group. The collected blood was evenly spread over pre-cleaned and pre-heated (40 °C) glass slides for each fish, slides prepared in triplicate were processed for the estimation of frequencies of micronuclei and other nuclear abnormalities.

Analysis of dumbbell nuclei and condensed/rounded nuclei: The phorate-induced dumbbell nuclei and condensed/rounded nuclei in erythrocytes of fish were analyzed by using Hoechst 33342 fluorescent dye (Sigma Aldrich, USA). The blood samples from each fish were transferred in EDTA coated vials using a heparinized syringe. After further processing, blood cells containing dumbbell nuclei and condensed/rounded nuclei were

observed and scored. At least 2000 cells were counted and the frequencies of dumbbell nuclei and condensed/rounded nuclei were calculated.

Statistical analyses : The data were statistically analyzed at $p < 0.05$. They were displayed as mean $\pm$ standard error mean (S.E.M.). The values of various parameters of treated and control groups were evaluated by employing one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. The data of studied parameters were analyzed by Statistical Package for the Social Sciences (SPSS) software (version 20.0, Chicago, IL, USA). Regression and correlation analyses were also established to find out the compact relationships between nuclear abnormalities and reactive oxygen species.

Results and Discussion

In comparison to control, ROS activity and CFS percentage significantly ($p < 0.05$) elevated in phorate treated groups, T1 (0.0375 mg l⁻¹) and T2 (0.075 mg l⁻¹) after 30 days (Fig. 1). Fold change in ROS activity was significantly ($p < 0.05$)

Fig. 1 : Representative graphs displaying a significant ($p < 0.05$) induction in ROS level (A) and the frequencies of erythrocytes showing fluorescent signals (CFS) (B) in phorate exposed *C. punctatus* of treated groups (T1 and T2) in comparison to control. The photographic images of erythrocytes showing ROS induced fluorescence in T1 (D) and T2 (E) groups in comparison to control (C) by using DCFH-DA dye (20 μ M) after 30 d. Values are mean $\pm$ SEM of 9 fish from each group. * indicated significance at $p < 0.05$ of treated against control.

enhanced 3.66 ± 0.28 -fold (T1) and 4.59 ± 0.28 -fold (T2) in phorate exposed erythrocytes of fish in comparison with the control (Fig. 1A). The frequencies of erythrocytes showing fluorescent signal (CFS), produced by ROS, were significantly ($p < 0.05$) increased 1.94-fold (T1) and 2.79-fold (T2) in phorate treated erythrocytes of fish in comparison with the control (Fig. 1B). The demonstrative photographic images of erythrocytes clearly display an elevation in ROS activities exhibiting fluorescent signal (CFS%) in phorate treated fish of T1 (Fig. 1D) and T2 (Fig. 1E) groups in comparison with unexposed group in a dose-dependent manner after 30 days (Fig. 1C). In context with this finding, an increase in reactive oxygen species was also described in fish *Cyprinus carpio* after long-term exposure to terbutryn (Velisek et al., 2011).

The frequencies of micronuclei (MN) significantly ($p < 0.05$) elevated in phorate-exposed erythrocytes of fish of both T1 and T2 in comparison with control in a dose-dependent manner after 30 days (Fig. 2). The frequencies of MN elevated 9.55-fold (T1) and 13.15-fold (T2) in phorate exposed erythrocytes of fish in

comparison with control (Fig. 2A). The photographic images evidently illustrate the phorate-induced micronuclei (MN) in erythrocytes of fish of T1 (Fig. 2C) and T2 (Fig. 2D) groups in comparison with the control (Fig. 2B) after 30 days of treatment. Similarly, the elimination of amplified DNA, chromosome or telomeric DNA damage and complete chromosome loss are the molecular phenomena that are instrumental in generation of MN, i.e., mini-circles of acentric chromatinic body (Podrimaj-Bytyqi et al., 2018). Notably, the main nucleus budded off or excluded the excess chromatin material or amplified DNA by certain active processes such as misrepair of DNA breaks and mal-segregation of chromosomes at anaphase to form a micronucleus (Fenech et al., 2011). This improper segregation of chromosome takes place due to inappropriate attachments of kinetochore-microtubules during the cell division (Godek et al., 2015). Several instances are there to record MN induction after exposure to different xenobiotics (Awasthi et al., 2018; Pandey et al., 2014; Yadav and Trivedi, 2009). The pesticides induced chromatin instability in terms of MN induction has also been reported in blood cells of humans (Coskun et al., 2011) and fish (Tiwari and Trivedi, 2019;

Fig. 2 : (A) Graph showing a significant ($p < 0.05$) induction in the frequencies of micronuclei (MN) in phorate treated groups (T1 and T2), in comparison to control after 30 d. The photographic images of erythrocytes showing MN, in T1 (C) and T2 (D) in comparison with control (B), by using 0.125% May-Grunwald's dye and followed by 5% Giemsa stain. Values are mean $\pm$ SEM of 9 fish from each group. * indicated significance at $p < 0.05$ of treated against control.

Trivedi et al., 2021).

The frequencies of notched nuclei (NN) and curved nuclei (CN) significantly ($p < 0.05$) elevated in phorate-exposed erythrocytes of fish of T1 and T2 in contrast to control after an exposure of 30 days (Fig. 3). Phorate-induced notched nuclei (NN) (Fig. 3B) and curved nuclei (CN) (Fig. 3C) in erythrocytes of fish of T2 (0.075 mg l^{-1}) group are markedly visible in comparison with the control (without NAs) (Fig. 3A). The frequency of notched nuclei (NN) significantly ($p < 0.05$) elevated 2.77-fold (T1) and 3.95-fold (T2) in phorate induced erythrocytes of test fish in comparison with control in a dose-dependent manner (Fig. 3D). Notably, notched nuclei (NN) generated due to incomplete invagination or noticeable deepness inside the nucleus and Ateeq et al., (2002) have also described this NA in blood cells of catfish, *C. batrachus*. This is pertinent to mention here that tubulin polymerization perturbations (TPP) induced aneugenic activity

was recognized as one of the main reasons for the generation of notched nuclei (Chondrou et al., 2018). Besides notched nuclei, the frequency of curved nuclei (CN) significantly ($p < 0.05$) increased 2.17-fold (T1) and 3.68-fold (T2) in phorate exposed erythrocytes of fish in comparison with control in a dose-dependent manner (Fig. 3E). Also, its related outcomes were described in freshwater fish *Australoheros facetus* after short-term exposure to endosulfan (Crupkin et al., 2013).

The microphotographic images of phorate exposed erythrocytes of fish evidently display the blebbed nucleus (BN) (Fig. 4B), nuclear bud (NBUD) (Fig. 4C), kidney-shaped nucleus (KN) (Fig. 4D), V-shaped nucleus (VN) (Fig. 4E) and nucleoplasmic bridge (NPB) (Fig. 4F) in test fish of T2 (0.075 mg l^{-1}) group in comparison with the control (without NAs) (Fig. 4A) after a treatment time of 30 days (Fig. 4).

Fig. 3 : Representative images of erythrocytes showing a notched nucleus (NN) (B) and curved nucleus (CN) (C) in phorate treated fish in comparison to control (without NAs) (A) by using 0.125% May-Grunwald's dye, followed by 5% Giemsa stain. Representative graphs illustrate a significant ($p < 0.05$) induction in frequencies of notched nuclei (NN) (D) and curved nuclei (E) in phorate exposed erythrocytes of fish of T1 and T2 as compared to control after 30 d. Values are mean $\pm$ SEM of 9 fish from each group. * indicated significance at $p < 0.05$ of treated against control.

The frequencies of nuclear anomalies (NAs) viz., blebbed nuclei (BN), nuclear buds (NBUD), kidney-shaped nuclei (KN), V-shaped nuclei (VN) and nucleoplasmic bridges (NPB) significantly ($p < 0.05$) elevated in phorate exposed erythrocytes of fish, *C. punctatus* of T1 (0.0375 mg l⁻¹) and T2 (0.075 mg l⁻¹) in comparison with the control in a dose-dependent manner after 30 d of exposure period (Fig. 5). The frequency of blebbed nuclei (BN) significantly ($p < 0.05$) increased 1.25-fold (T1) and 2.75-fold (T2) in phorate exposed erythrocytes of fish in comparison with the control (Fig. 5A). At the cellular level, amplified DNA or chromatin material slightly localizes upon the periphery of the nucleus and is termed as blebbed nuclei (BN). This class of NAs is identified as a minor evagination of amplified DNA or affected chromatin material through the process of exocytosis. After getting surrounded by a membrane, the evagination becomes BN (Fenech, 2006). In context with BN, Weldetinsae *et al.* (2017) have emphasized that the phenomenon acknowledged as breakage-fusion-bridge cycle was primarily responsible for bulging of amplified DNA or chromatin from the nucleus. Apart from blebbed nuclei, the frequency of kidney-shaped nuclei (KN)

was significantly ($p < 0.05$) increased 1.00-fold (T1) and 2.52-fold (T2) in phorate exposed blood cells, especially erythrocytes of fish in comparison control (Fig. 5C). Previously, several studies have reported the elicited frequency of kidney-shaped nuclei in different pesticides induced fish species (Ahmad and Ahmad, 2016; Marques *et al.*, 2014). Successively, the frequency of V-shaped nuclei was also significantly ($p < 0.05$) elevated 1.99-fold (T1) and 5.13-fold (T2) in phorate exposed erythrocyte of fish in comparison with the control (Fig. 5D). As well as, the findings are also in agreement with that of da Silva Souza and Fontanetti (2006) who have documented NAs in blood cells of *Nile tilapia* intoxicated with refinery effluent in contaminated aquatic environments. Moreover, the frequency of nuclear buds (NBUD) significantly ($p < 0.05$) elevated 1.94-fold (T1) and 3.37-fold (T2) in phorate induced erythrocytes of test fish in comparison with control (Fig. 5B). Further, the frequency of nucleoplasmic bridge (NPB) significantly ($p < 0.05$) increased 1.55-fold (T1) and 3.42-fold (T2) in phorate exposed erythrocytes of fish, *Channa punctatus* in comparison with the control in a dose-dependent manner after 30 d (Fig. 5E). In the present study, the frequency of

Fig. 4 : Demonstrative photographic images of erythrocytes showing blebbed nucleus (BN) (B), nuclear bud (NBUD) (C), kidney-shaped nucleus (KN) (D), V-shaped nucleus (VN) (E) and nucleoplasmic bridge (NPB) (F) in phorate exposed fish in comparison to control (without any NAs) (A) by using 0.125% May-Grunwald's dye, followed by 5% Giemsa stain, after 30 d.

both NBUD and NPB were found significantly ($p < 0.05$) elevated in erythrocytes of phorate exposed fish (T1 and T2) in comparison with the control in a dose-dependent mode. Correspondingly, Serrano-García and Montero-Montoya, (2001) have also observed induction in the frequency of chromatin buds (CHB) in lymphocytes of smokers exposed to drugs. Actually, NBUD is formed through major evagination of euchromatin or amplified DNA bounded by membrane, while NPB originates by partial segregation of genetic material or euchromatin or partial karyokinesis (Garaj-Vrhovac et al., 2008). These nuclear anomalies represent incomplete evagination of certain parts of genomic DNA and misrepair or telomere end-fusions. Further, it is well established that NBUD is generated by evagination of

chromatin material (from nucleus) consisting 44% of telomeric DNA exclusively and 10% of both telomeric and centromeric DNA (Lindberg et al., 2007). Thus, it can be inferred that both NBUD and NPB are generated due to failure of DNA repair and inactivation of essential mechanisms required for removal of misrepair complexes. Marques et al. (2014) and da Rocha et al. (2011) elaborated the induction of nuclear anomalies in deltamethrin exposed *Anguilla anguilla* and in potassium dichromate exposed *Oreochromis niloticus*, respectively.

Evidently, the characteristic photographic images demonstrate phorate-induced dumbbell nucleus (DN) in erythrocytes of test fish of T2 (0.075 mg l⁻¹) group (Fig. 6B) in

Fig. 5 : Demonstrative graphs showing a significant ($p < 0.05$) induction in frequencies of blebbed nuclei (BN) (A), nuclear bud (NBUD) (B), kidney-shaped nuclei (KN) (C), V-shaped nuclei (VN) (D) and nucleoplasmic bridge (NPB) (E) in phorate exposed erythrocytes of fish of T1 and T2 in comparison to control after 30 d. Values are mean $\pm$ SEM of 9 fish from each group. * indicated significance at $p < 0.05$ of treated against control.

comparison with the control (without any NAs) (Fig. 6A) after 30 days of treatment (Fig. 6). The frequency of dumbbell nuclei (DN) significantly ($p < 0.05$) increased 1.70-fold (T1) and 3.91-fold (T2) in phorate exposed erythrocytes of fish in comparison with the control in a dose-dependent manner after 30 days of exposure period (Fig. 6C). Increased frequencies of dumbbell nuclei have also been reported by Falfushynska *et al.*, (2013) in a fresh water bivalve, *Anodonta anatina* against its exposure to trace metals.

The frequency of condensed/rounded nuclei (CRN) were significantly ($p < 0.05$) induced in phorate exposed erythrocytes of fish of T1 and T2, in contrast, to control after 30 days of treatment time (Fig. 7). Notably, blood cells containing CRN illustrate early stage of apoptosis. The frequency of condensed/rounded nuclei (CRN) elevated 2.78-fold (T1) and 4.80-fold (T2) in phorate treated fish in comparison with the control (Fig. 7A). It is well known that CRN, a biomarker of early apoptosis, is recognized as a whole chromatin material inside the nucleus with an intact membrane. Its frequency was significantly ($p < 0.05$) augmented in phorate exposed erythrocytes of fish of treated groups, in contrast, to control in a dose-dependent manner. Evidently, the brighter fluorescence was observed due to nuclear condensation

in phorate exposed erythrocytes of *C. punctatus* of treated groups (T1 and T2) with respect to the unexposed group (Fig. 7). Similarly, Mai *et al.* (2010) have also reported the induction of apoptosis in terms of condensed or fragmented nuclei in tilapia blood cells. Markedly, the representative photographic images exemplify phorate-induced condensed/rounded nuclei (CRN) in erythrocytes of test fish of T1 (Fig. 7C) and T2 (Fig. 7D) in comparison with the control (Fig. 7B). Subsequently, the genetic material becomes more susceptible for damage. Further, enhanced membrane permeability also facilitates the transport of caspase-independent factors, particularly apoptosis inducing factor (AIF) and mitochondrial endonuclease G (endo G) to the nucleus via cytoplasm (Candé *et al.*, 2002). In the nucleus, these factors become instrumental for condensation and fragmentation of chromatin or DNA strands which engenders various nuclear anomalies (Tebourbi *et al.*, 2011). Eventually, the aforesaid data related to nuclear abnormalities were significantly ($p < 0.05$) increased in phorate exposed erythrocytes of fish in comparison with the control in a dose-dependent manner. Moreover, Walia *et al.* (2013) and Noor and Zutshi (2018) have also reported induction of NAs in blood cells of fish, *Labio rohita*.

Fig. 6 : Photographic images showing the dumbbell nucleus (DN) (B) in phorate exposed erythrocytes of fish in comparison to control (without DN) (A) by using Hoechst 33342 (1.5 mg ml⁻¹) fluorescent dye. (C) A graph showing a significant ($p < 0.05$) induction in frequencies of dumbbell nuclei (DN) in phorate induced erythrocytes of fish of T1 and T2 in comparison to control after 30 d. Values are mean $\pm$ SEM of 9 fish from each group. * indicated significance at $p < 0.05$ of treated against control.

Fig. 7 : (A) Graph showing a significant ($p < 0.05$) induction in frequencies of condensed/rounded nuclei (CRN) in phorate exposed erythrocytes of fish of T1 and T2 in comparison to control after 30 d. The photographic images of erythrocytes showing CRN (by arrows) in T1 (C) and T2 (D) in comparison to control (B) by using Hoechst 33342 fluorescent dye (1.5 mg ml^{-1}) in phorate exposed fish *C. punctatus*. Values are mean $\pm$ SEM of 9 fish from each group. * indicated significance at $p < 0.05$ of treated against control.

The correlation analysis illustrates a prominent correlation between the phorate induced ROS and consequent nuclear abnormalities in erythrocytes of *Channa punctatus* after 30 days of treatment. Notably, the correlation analysis also displays the compact relationships among the phoarte induced ROS and various NAs with greater values of coefficient of correlation (R) in erythrocytes of treated fish. Comparatively, the highest correlation coefficient was found for MN and the lowest for VN. Thus, the values of correlation coefficients clearly illustrate the sensitivity of nuclear anomalies as a predictor of phorate toxicity in the test fish. Accordingly, the pattern of their sensitivity was stipulated as follows; MN > NN > CN > CRN > NBUD > NPB > BN > DN > KN > VN.

The chronic exposure of phorate generates cellular stress in fish. This culminates into genotoxicological impairments

like MN and other NAs. Thus, the extent of expression of nuclear abnormalities can be amply employed in molecular bio-monitoring of stressed aquatic regimes. Further, their estimation is cost effective, viable, precise and with lesser involvement of high-throughput technologies. Present findings, too, successfully establish their role as sensitive biomarkers in monitoring the health status of fishes inhabiting aquatic regimes contaminated with phorate.

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Add-on Information

Author's contribution : **S. P. Trivedi:** Conceptualization, Experimental designing, final editing of the MS & proof correction; **A. Ratn :** Execution of the Experiment and analysis of nuclear abnormalities; **Y. Awasthi :** Photography and estimation of ROS and nuclear abnormalities; **N. Gupta:** Statistical analysis of the data and critical evaluation of the MS; **M. Kumar:** Supervision of the experiment & preparation of the preliminary manuscript; **A.Trivedi:** Plotting of Graphs, tabulation and overall analysis of the data.

Research content : The research content of manuscript is original and has not been published elsewhere.

Ethical approval : For entire experimentation and euthanization of fish, necessary guidelines issued by the Institutional Animal Ethics Committee (IAEC, Registration No. 1861/GO/Re/S/16/ CPCSEA) were followed.

Conflict of interest : The authors declare that there is no conflict of interest.

Data from other sources : Not applicable

Consent to publish : All authors agree to publish the paper in *Journal of Environmental Biology*.

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