MEDWIN PUBLISHERS
Committed to Create Value for Researchers

International Journal of Oceanography & Aquaculture
ISSN: 2577-4050

Monitoring Stress Biomarkers Anomalies of Grass Carp (*Ctenopharyngodon idella*) Valenciennes, 1844) Post-Exposure to Chlorpyrifos

Khan N[1]*, Sultan A[1] and Rahman IU[2]

[1]Department of Biology, University of Haripur, Pakistan

[2]Department of Zoology, University of swabi, Pakistan

Short Communication

Volume 7 Issue 3

Received Date: August 02, 2023

Published Date: August 24, 2023

DOI: 10.23880/ijoac-16000254

***Corresponding author:** Naqash Khan, Department of Biology, University of Haripur, Haripur, Khyber Pakhtunkhwa, Pakistan, Tel: 03109661266; Email: knaqash649@gmail.com

Keywords: Aquatic Ecosystem; Organophosphorus; Fish; *Ctenopharyngodon idella*; Chloropyrifos; Haematological Indices

Abbreviations: MN: Micro Nucleus; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration.

Introduction

Releasing of contaminants to any environmental medium from any source which are capable of causing harm to man or any other living organisms ends in environmental pollution. Environmental pollution exists from the beginning of the life but with the passage of time it becomes a worldwide issue that threatens the surrounding living organism [1].

A pesticide is a substance or it is a mixture of substances that made for the preventing, destroying, repelling, or decreasing the damage of any pest that cause to the anything's [2]. Contamination of aquatic ecosystem by pesticides is a serious problem and fishes are more frequently exposed to these pollutants and may be taken inside the body via gills, skin and contaminated foods [3]. Chlorpyrifos is a well-known insecticide of the organophosphorus family. It was registered in 1965 and still used in more than 80 countries to prevent and reduce insect damage to agricultural products, as well as on turf, and to control termites and house hold pests.

The exposure of aquatic organisms to various stressed and pollutants practically insecticide in their environment generally causes rapid changes in various hematological parameters of fish. Hematological parameters are important for toxicological research and have been widely used in environmental monitoring, and as indicators of disease and environmental stress. Fish are widely used as bio indictor to evaluate the health of aquatic ecosystems because pollutants build up in the food chain and are responsible for adverse effects and death in the aquatic system [4].

DNA damage by chlorpyrifos in *Channa punctatus* using micro nucleus (MN) and comet assays has also been investigated by Ali, et al. Chlorpyrifos after 24h showed decrement in RBC (-72.43%) and hemoglobin (-18.35%) and an increment in WBC (+57.94%) compared to controls in *Cyprinus carpio* [5]. Therefore, the main purpose of the present study was to investigate the hematologicall alterations in the freshwater fish, *C. idella* after in vivo acute exposure to various doses of chloropyrifos.

Materials and Methods

Chemical, Test Animal and Study Duration

Chemical selected for the current study was insecticide named as chloropyrifos which is the most important part of modern agricultural setup because it is used as extensively against the herbs although it extensive utilization has adverse impact on the surrounding ecosystem. In the current study, chlorpyrifos insecticide was introduced at sub lethal concentration i.e. 0.03 μL^{-1}, for a period of 3, 6,9,12 and 15 days respectively against *C. idella*. Fingerlings of grass carp (3.23 ± 0.34g) were packed in oxygenated bags and transported

from Mardan and Peshawar carp hatcheries to Fisheries and Aquaculture research lab, Department of Zoology, University of Swabi. Before experimentation fish were acclimatized for two weeks in aquarium having tap water, 500L and were fed properly with commercial pellets carp diet. Air pumps were installed in aquarium for purpose to aerate the aquarium water so that all the water quality parameters should be adjusted to normal range particularly dissolved oxygen which is important parameters that plays critical role in fish survival. The hardness and pH of water were 250 mg $CaCO_3$ L^{-1} and 7.4 ± 0.01, individually. The medium utilized was filtered and the degrees of NH_4, NO_2 and NO_3 in the water recorded to be inside 0.1, 0.1 and 20 mg L^{-1}, separately. After stipulated duration of acclimatization, randomly fish were transported to experimentation aquarium having 100L water. These tanks were also having the air pumps to aerate the water and to maintain the aquarium environment suitable for fish. During the experiment, grass carp behavior rate were also recorded.

DNA Damage Analysis

DNA damage was analyzed in fish blood through the process of comet assay according to Singh, et al. with minor modifications. Fish blood were immobilized on a clean clear slide through agarose gel, then were lysed with lysis buffer, stained with ethidium bromide and analyzed through TriTek Comet Score that is classified into five categories (measured) starting from Type 0 (undamaged) to Type IV (complete damage). Comet Software was used to measure the comet tail length of damaged cells and cumulative tail length of all examined cells (n=50 per replicate).

Biochemical Indices Analysis

Cortisol hormone levels contents were assessed using the radioimmunoassay methods as described by Waring CP, et al. [6]. Electrolytes and glucose levels were assessed using the flame emission method according to Lerner, et al. The concentrations of Cl^-, Ca^{2+}, and glucose were determined using a semi-automated clinical chemistry analyzer Pictus B (Diatron, Hungary).

Blood Analysis

The fresh whole blood collected in EDTA tubes was instantly subjected to analyse the blood indices through a hematological analyzer (PocH-1001V, Sysmex Corp., Kobe, Japan).

Statistical Analysis

One-way ANOVA test (IBM SPSS Statistics 20) was used to determined significant differences between the untreated and treated variables.

Results

Parameters including hematology, biochemistry and DNA damage of grass carp were observed against the specific sub lethal dose of chlorpyrifos insecticide for various time periods. Behavior of fish was also under observation throughout the experimentation.

Behaviorally Observations

Behavior of fish were observed regularly during the experimentation and noted that chlorpyrifos poisoning in fish ends in high rate of breathing due to suffocation that was observed by maximum frequency of opercular movement, loss of body balance, includes erratic and jerky swimming, frequent surfacing, becoming exhausted and lethargic and bleeding at the base of the eyeballs were documented which in in line with the findings of Khan [7].

Hematological Indices Analysis after 3 Days

The hemoglobin concentration (g dl^{-1}) mean value 9.8 ± 0.680 in comparison to the mean value of 12.12± 0.360 for the control group showed a significant declined ($p < 0.05$) after the exposure for 3 days. Similarly declined in concentration of red blood cells (millcm^{-1}) 1.82 ± 0.104 were observed in comparison of control group having mean value of 2.92±0.088. In stress environment, the total leukocyte counts (cmm^{-1}) of *C. idella* were significant incremented ($p<0.05$) against the chlorpyrifos having mean value 61350±17065.1 in comparison with control group mean value of 8100±2066.39 respectively, while significant decrease ($p<0.05$) in platelets counts (lakscu-1) 5666 ± 2081.66 were noticed in comparison with the reference group 8100±2066.39. Except the mean concentration of MCV(Mean corpuscular volume, fl^{-1}) 97.6±7.879, the mean concentration of MCH (Mean corpuscular hemoglobin, pg^{-1}) and MCHC (Mean corpuscular hemoglobin concentration, gdL^{-1}) of treated group, 54.1±1.242 and 55.6±3.51 showed significant elevation ($p<0.05$) in contrast to control group mean value 47.8±0.665 and 47.3±0.577 respectively. To cope with the stressful surroundings as imposed by the toxicity of chlorpyrifos the *C. idella* increased the immunity system that is denoted by the increase in the mean concentration of lymphocytes (%) 87.3±6.35 in comparison with control group mean concentrations as 76.6±2.88, while significant decline ($p<0.05$) in concentration of neutrophiles, monocytes and eosinophiles (%) were recorded 1±1.154, 6.3±2.30 and 4.6 ± 2.88 respectively in contrast to untreated group mean concentrations as 10±2.645, 7.6±2.309 and 6±1.73 respectively while no data were recorded for the basophiles in all treated groups.

Hematological Indices Analysis after 6 Days

Similarly the hemoglobin concentration (g dl^{-1}) mean value 10.03± 0.383 in comparison to the mean value of 12.12±0.360 for the control group showed a declined after the exposure for 6 days. Similarly declined in concentration of red blood cells ($millcm^{-1}$) 1.8±0.166 were observed in comparison of control group having mean value of 2.92±0.088 while non-significant difference were seen in the total leukocyte counts (cmm^{-1}) of *C. idella* against the chlorpyrifos having mean value 81600±6077.00 in comparison with control group mean value of 8100±2066.39 respectively. Significant decrease ($p<0.05$) in platelets counts ($lakscu^{-1}$) 5666±1154.7 were noticed in comparison with the reference group 8100±2066.39. The mean concentration of MCV(Mean corpuscular volume, fl^{-1}), MCH (Mean corpuscular hemoglobin, pg^{-1}) and MCHC (Mean corpuscular hemoglobin concentration, gdL+) of treated group 89±3.360, 53±3.36 and 59.6±0.577 showed variations of small ranges in contrast to control group mean value 101.03±1.184, 47.8±0.665 and 47.3±0.577 respectively. Increased in the mean concentration of lymphocytes (%) 82.6±2.516 and Monocytes (%) 8.66±2.516 in comparison with control group mean concentrations as 76.6±2.88 and 7.6±2.309 respectively were noticed. While significant decline ($p<0.05$) in concentration of neutrophiles and eosinophiles (%) were recorded 3.33±2.88 and 5.3±1.154 respectively in contrast to untreated group mean concentrations as 10±2.645 and 6±1.73 respectively while no data were recorded for the basophiles in all treated groups.

Hematological Indices Analysis after 9 Days

After exposure duration of 9 days, decline in the hemoglobin concentration (g dl^{-1}) mean value 10.7±0.665 in comparison to the mean value of 12.12±0.360 for the control group were recorded. Similarly declined in concentration of red blood cells ($millcm^{-1}$) 1.91±0.150 were observed in comparison of control group having mean value of 2.92± 0.088 while significant difference ($p < 0.05$) were seen in the total leukocyte counts (cmm^{-1}) of *C. idella* against the chlorpyrifos having mean value 64866±13855.08 in comparison with control group mean value of 8100±2066.39 and significant decrease ($p<0.05$) in platelets counts ($lakscu^{-1}$) 10333±13576.94 were noticed in comparison with the reference group 8100±2066.39. The mean concentration of MCV (Mean corpuscular volume, fl^{-1}) showed significant variation 89±3.874 in contrast to control group mean concentration 101.03±1.184 while MCH (Mean corpuscular hemoglobin, pg^{-1}) and MCHC (Mean corpuscular hemoglobin concentration, gdL^{-1}) of treated group 56.3±1.040 and 63.3±4.932 showed variations of small ranges in contrast to control group mean value 47.8±0.665 and 47.3±0.577 respectively. Mean concentration of lymphocytes (%) 89.6±2.51 in comparison with control group mean concentrations as 76.6±2.88 increase to adjust the body immunity with the surrounding environment While significant decline ($p<0.05$) in concentration of neutrophiles, monocytes and eosinophiles (%) were recorded 1.3±0.577, 4.6±0.577 and 4.3±1.52 respectively in contrast to untreated group mean concentrations as 10±2.645 and 6±1.73 and 6±1.732 respectively while no data were recorded for the basophiles in all treated groups.

Hematological Indices Analysis after 12 Days

After exposure duration of 12 days, the hemoglobin concentration (g dl^{-1}) mean value 9.1± 0.346 in comparison to the mean value of 12.12±0.360 for the control group showed a significant declined ($p<0.05$). Likewise declined in concentration of red blood cells ($millcm^{-1}$) 2.06± 0.1552 were observed in comparison of control group having mean value of 2.92± 0.088 while significant decline; $p < 0.05$,were seen in the total leukocyte counts (cmm^{-1}) of *C. idella* against the chlorpyrifos having mean value 59733± 15992.92 in comparison with control group mean value of 8100±2066.39 and significant decrease ($p<0.05$) in platelets counts ($lakscu^{-1}$) 6666±4618.802 were noticed in comparison with the reference group 8100±2066.39. The mean concentration of MCV(Mean corpuscular volume, fl^{-1}), MCH (Mean corpuscular hemoglobin, pg^{-1}) and MCHC (Mean corpuscular hemoglobin concentration, gdL^{-1}) of treated group concentrations 105.9±8.075, 47.5±2.157 and 45.1±5.157 showed non-significant variation in contrast to control group mean concentration 101.03±1.184, 47.8±0.665 and 47.3±0.577 respectively. Mean concentration of lymphocytes and eosinophiles (%) 83.6±3.577 and 8.6±2.516 in comparison with control group mean concentrations as 76.6±2.88 and 6±1.732 increase to adjust the body immunity with the surrounding environment While significant decline ($p<0.05$) in concentration of neutrophiles and monocytes (%) were recorded 7.6±0.577 and 5.6±0.577 respectively in contrast to untreated group mean concentrations as 10±2.645 and 7.6±2.309 respectively while no data were recorded for the basophiles in all treated groups.

Hematological Indices Analysis after 15 Days

The hemoglobin concentration (g dl^{-1}) mean value 10.2± 0.655 in comparison to the mean value of 12.12±0.360 for the control group showed declined after the exposure for 15 days. Similarly declined in concentration of red blood cells ($millcm^{-1}$) 1.87±0.1357 were observed in comparison of control group having mean value of 2.92±0.088 while significant inclined ($p<0.05$) in concentration were seen in the total leukocyte counts (cmm^{-1}) of *C. idella* against the chlorpyrifos having mean value 32133±3716.62 in comparison with control group mean value of 8100±2066.39

and significant decrease ($p<0.05$) in platelets counts ($lakscu^{-1}$) 10333±2516.61 were noticed in comparison with the reference group 8100±2066.39. The mean concentration of MCV (Mean corpuscular volume, fl^{-1}) and MCH (Mean corpuscular hemoglobin, pg^{-1}) of treated group 115.7±4.80 and 54.9±2.218 were significantly increased ($p<0.05$) in contrast to control group concentration 101.03±1.184 and 47.8±0.665 while MCHC (Mean corpuscular hemoglobin concentration, gdL^{-1}) of treated group concentrations 47.6±1.154 showed non-significant variation in contrast to control group mean concentration respectively. Mean concentration of lymphocytes, monocytes and eosinophiles (%) 82±11.357, 12.6±0.577 and 7±01 in comparison with control group mean concentrations of control group as 76.6±2.88, 7.6±2.309 and 6±1.732 increase to adjust the body immunity with the surrounding environment While significant decline ($p<0.05$) in concentration of neutrophiles (%) were recorded 8.3±1.527 respectively in contrast to untreated group mean concentrations as 10±2.645 respectively while no data were recorded for the basophiles in all treated groups.

Electrolyte Concentration

Electrolyte concentrations (K^+, Cl^-, Na^+) along with glucose concentration were documented against the chlorpyrifos specific concentration and were compare with the control group and it was observed that all the parameters were altered in a way that maximum incremented ($p<0.05$) concentration were observed against the chlorpyrifos. Chloride concentration was effected maximum that unveiled the alteration in osmoregulation of fish because of the toxic surrounding followed by glucose, potassium and sodium.

Discussion

Assessing contaminants toxicity in fish is of extraordinary concern because of their expected unfavorable effect on human being after utilization in food. Hence toxicity study are fundamental for decide sensitivity of animals and human beings to poisons and furthermore helpful for assessing the level of harm to target organs and the consequent physiological, biochemical and behavioral disorders [8]. In the current experimentation, during toxicity period, different behaviorally irregularities like expanded opercula, mucous discharge, and jerky development, drifting on the sides, hypersensitivity showing violent erratic and quick swimming and so on have been seen which demonstrates the harmful impact of chlorpyrifos on central nerves framework and cardiovascular framework [9].

Chemical contamination of aquatic ecosystem can influence the balance of fish and can be utilized as a index of toxic stress [10]. By adapting various behaviorally movements, fish attempt to decrease the impacts of pesticides enter in to the body from the surrounding medium or to limit the harm of their body tissues. In the current examination, treated fish documented disquiet and lazy swimming which is as per the report of Bradbury and Coats. As indicated by Marler and Hamilton, changes in behavior of Labeo rohita may be because of the impact of pesticides on the central nervous system or the aggravations in physiological component. Comparable behavioral adaptations have likewise been accounted for in *Cyprinus carpio* against Diazinon and in Labeo rohita against cypermethrin and diazinon toxicity.

Among physiological disturbance against the toxicity, hematological indices are viewed as likely biomarkers of toxicity as incline or decline in the different hematological indices can be observed. In comparison with the control group, the hematological indices of *C. mrigala* exposed to 0.815 mg/L and 1.63 mg/L of diazinon for 30 days was recoreded a huge decline in RBC, Hb, HCT, MCV, MCH and WBC count. The decrementing in in RBC, WBC counts and upsides of other hematological indices in *C. mrigala* can be credited to concealment of hematopoietic system of the fish, brought about by longterm toxicity to diazinon. These outcomes are reliable with the findings of different examinations exploring hematological indices of various fish species exposed to other organophosphate pesticides as well similarly as with the current observation of chlorpyrifos against grass carp. In this association, changes in hematological lists were seen after exposure to malathion in *C. gariepinus*, phosalone in *O. mossambicus* and trichlorfon in *C. carpio*. Comparative decline in RBC count, WBC count, hemoglobin and hematocrit levels have been accounted for in male brood stock, *Rutilus frisii kutum* and grass carp, *Ctenopharyngodon idella* after long term exposure to sub-deadly centralizations of diazinon. Additionally detailed declined in Hb, RBC and WBC count and expanded upsides of MCV and MCH after diazinon exposure in monster sturgeon (huso) and African catfish, *C. gariepinus* separately.

Red blood cells concentration was declined in fish exposed to toxicant surroundings. Downturn in the erythrocyte profile ends in oxygen deficiency in the body because of gill harm due to contaminants [11]. Restraint of erythropoiesis and expansion in the rate of erythrocyte obliteration in hematopoietic organs is the reason for decline in RBC count. In the current study, the critical declined in RBCs and hemoglobin content could have resulted because of the declining of the oxygen content of the water because of the presence of chlorpyrifos in the surrounding ecosystem. Leucocytes are engaged with the regulations of immunological capacity and their concentration increment as defensive reaction in fish against toxic media. Such an expansion leucocyte count (TLC) happens by the increment in lymphopoiesis and additionally upgraded release of

lymphocytes from lymphoid tissues. Fink and Salibian revealed that WBC increment could in connection of an induced proliferation because of the insecticide toxicity.

In the current study Comet test was applied to assess the DNA strand breaks in the peripheral blood erythrocytes of common carp presented in vivo to sub-lethal dose of chlorpyrifos insecticide. Present observations showed that exposure of fish to chlorpyrifos prompted fundamentally higher DNA harm in blood when contrasted with control group.

Acknowledgment

The author is greatly acknowledging the Department of Biology, University of Haripur, Pakistan for providing experimental facilities.

Conflict of interest

All the authors confirmed that the content of this manuscript has no conflict of interest.

References

1. Skenderovic I, Kalac B, Becirovic S (2015) Environmental pollution and waste management. Balkan Journal of Health Science 3(1): 1-10.

2. Eldridge BF (2008) Pesticide application and safety training for applicators of public health pesticides. California Depart ment of Public Health, Vector-Borne Disease Section, 1616 Capitol Avenue, MS7307, P.O. Box 997377, Sacr amento, CA.

3. Ling XP, Zhang YH, Lu YH, Huang HQ (2011) Superoxide dismutase, catalase and acetyl cholinesterase: biomarkers for the joint effects of cadmium, zinc and methyl parathion contamination in water. Environmental Technology 32(13-14): 1463-1470.

4. Farkas A, Salanki J, Specziar A (2002) Relation between growth and the heavy metal concentration in organs of bream *Abramis brama* L. populating Lake Balaton. Archives of Environmental Contamination and Toxicology 43(2): 236-243.

5. Ramesh M, Saravanan M (2008) Haematological and biochemical responses in a freshwater fish Cyprinus carpio exposed to chlorpyrifos. International Journal of Integrative Biology 3(1): 80-83.

6. Waring CP, Moore A (2004) The effect of atrazine on Atlantic salmon (Salmo salar) smolts in fresh water and after sea water transfer. Aquat Toxicol 66(1): 93-104.

7. Khan N, Khan A, Sultan A, Ullah K, Ali A (2023) Hematological Alteration Induced in the Fish Grass Carp (*Ctenopharyngodon idella*) Exposedto Bisphenol A (BPA) Compound. J Fisheries Livest Prod 11(6): 429.

8. Rainer F, Pauly D (2011) Species of Clarias in fish base.

9. Antychowicz J, Szymbor E, Roszkowski J (1979) Investigations upon the effects of some pesticides on carp (*Cyprinus carpio*). Bull Vet Inst In Pulawy 23(3-4): 124-130.

10. Dobsikova R, Velisek J, Wlasow T, Gomulka P, Svobodova Z, et al. (2006) Effects of cypermethrin on some haematological, biochemical and histopathological parameters of common carp (*Cyprinus carpio*). Neuro Endocrinol Lett 27(2): 91-95.

11. Drastichova J, Svoboda M, Luskova V, Zlabek V (2001) The effect of diazinon on haematological indices of common carp (*Cyprinus carpio L*). Acta Vetrinary Bruno 70: 457-465.