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HEMATOPATHOLOGY AND HISTOLOPATHOLOGY ALTRATION EXPOSURE PEROIDS OF $C_4H_{10}NO_3PS$ IN FRESHWATER *Channa punctata*

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The current examination surveys the intense poisonousness and conduct changes due to Acephate ($C_4H_{10}NO_3PS$), an organophosphate pesticide on *Channa punctata* (*C. punctata*). The sublethal centralization of $C_4H_{10}NO_3PS$ is 910 mg/L. In the current examination the changes in the hematological profiles were explored in *C. punctata* after presentation to deadly and sublethal exposures of $C_4H_{10}NO_3PS$. The estimations of various blood parameters after toxicant introduction and percent changes over control were introduced. Toxicants primarily follow up on circulatory framework and show significant effect on blood parameters. Subsequently, our current examination uncovered the impact of pesticide poisonousness on blood parameters. In contemplates, we have watched the critical changes in blood parameters after presented to 1 day deadly, 1 sublethal, multi day sublethal and 10 sublethal groupings of $C_4H_{10}NO_3PS$. In the current investigation the RBC checks, WBC tallies, Hb, and PCV levels were diminished essentially ($p < 0.05$) toxicant uncovered fish when contrasted with control fish. The MCV, MCH and MCHC levels were expanded in toxicant uncovered fish when contrasted with control fish. Likewise the Glucose, TL, AST and ALT levels were essentially expanded after presentation of $C_4H_{10}NO_3PS$ however the TP esteems were diminished altogether.

Keywords: Acephate; *Channa punctata*; LC_{50}; hematological parameters.

1. INTRODUCTION

Formers were used agricultural pesticides to protect their crops and animals from pests and diseases in contemporary agriculture and are biologically active chemical substances. These pesticides are carried into the aquatic environment by surface runoff from sites of application, where they enter the organisms through food webs and also through contact in water. Therefore, the health of the aquatic ecosystem is negatively affected because they serve as an ultimate sink for these pesticides. Currently the aquatic environment is beneath danger due to the increase of pesticide pollution by the human activities and causing high risk to non-target organisms [1,2]. In addition, an increase in agricultural practices in order to overcome the needs of increasing population the degradation of aquatic system is a worldwide phenomenon. Fish live in water and extremely close contact with their environment, and

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consequently they are very susceptible to any physicochemical changes which may be reflected in their blood parameters [3]. The employing of hematological techniques in fish culture has growing importance for toxicological research, environmental inspecting and fish health conditions. Several studies have been carried out on the hematological changes in fish as a result of pesticides by [4,5,6] reported that the blood parameters of diagnostic significance are erythrocyte and leukocyte differential counts would readily respond to incidental factor such as physical stress and environmental stress caused by water quality is affected by toxicants, any physiological changes will be reflected in the values of one or more of the hematological parameters.

Fishes exhibit hematological changes due to the direct exposures of toxicants such as metals, pesticides and industrial effluents, not only after laboratory exposure, but also when the exposure occurs in the field of natural sources. A thin epithelial membrane separates fish blood from the water and any adverse change in the water body is revealed in the blood [7]. Furthermore, it should be reported that hematological indices are of different sensitivity to different environmental factors and chemicals. Previous hematological studies of pollutants brought to the knowledge that erythrocytes are the major and reliable indicators of various sources of stress [8]. Blood cell responses are inept indicators of changes in the internal and/ or exterior environment of the animals. The exposure of fish to chemical pollutants can either induce an increase or decrease in the hematological levels. The toxicant induced changes mainly depend on the fish species, age, the cycle of the sexual maturity of spawners and diseases. It is a path physiological indicator of the whole body function and therefore blood parameters are important in diagnosing the structural and functional status of fish exposed to a toxicant. A number of hematological indices such as haemoglobin (Hb), hematocrit (HCT), red blood cells (RBCs), and White blood cells (WBCs) and so on, have been used as indicators of pesticide pollution in the aquatic environment.

Blood parameters are regarded as good physiological indicators of the whole body conditions and therefore can be exercised in diagnosing the structural and functional status of fish exposed to toxicants [9,10]. They have been increasingly employed in environmental monitoring programs to indicate physiological changes due to toxicants. However, the knowledge on the fish hematology still needs to be expanded, to provide data for different species and its exposure to the different toxicants. Such a study would be useful as the exposure of fish to various diverse types of chemical agents may induce differential changes in hematological variables. The values of hematocrit, hemoglobin, and the number of erythrocytes are indicators of toxicity with a wide potential for application in environmental monitoring and toxicity studies in aquatic animals [11]. In addition the determination of the packed cell volume (PCV), and obtaining total erythrocyte counts and red blood cell indices, such as mean cell volume, mean corpuscular hemoglobin concentration, and mean corpuscular hemoglobin, all can be useful in diagnosing disease. The PCV varies within and between species and seems to correlate with the normal activity level of the fish. In this study, the effect of the $C_4H_{10}NO_3PS$ on the hematological and biochemical profile of freshwater teleost fish, *C. punctata* was studied.

2. MATERIALS AND METHODS

The healthy freshwater fish *C. punctata* (length, 10 ± 0.9 cm; weight, 10 ± 0.8 g) fingerlings were collected from the private fish ponds of Kuchipudi village, Guntur district in Andhra Pradesh, India. The fish were maintained in large circular plastic tubs with reconstituted water for 10-15 days under standard laboratory conditions for acclimatization. The water was constantly aerated with rich oxygen in static system. The fish were fed with rice bran and commercial fish pellets once in a day after cleaning the faecal matter and other waste materials from the tub to avoid accumulation of ammonia and methane gas. The fish specimens were anesthetized with methane sulfonate (MS- 222, Sigma Chemical Co, USA) and 1 mL of blood was obtained by caudal vein puncture and placed in glass tubes containing EDTA (Sigma Chemical Co, USA), while the fish were sedated. Plasma was obtained by centrifugation of blood at 3000 rpm for 15 min and non-hemolysed plasma was stored in a deep freezer for further biochemical analyses. From the collected blood sample RBC, WBC and Hb were determined as follows: Neubauer hemocytometer was used to determine RBC and WBC counts. Care was taken to avoid trapping of air bubbles. The RBC lying inside the five small squares was counted under high power (40X) of light microscope. The following formula was used to calculate the number of RBC per mm^3 (µL) of the blood sample:

Number of RBC/mm^3= (N × dilution)/ area counted × depth of fluid.

Pesticide: The commercial grade formulations of $C_4H_{10}NO_3PS$ 75% SP an organophosphate pesticide is used as a toxicant in the present experiment and

showed of 2D structure and IUPAC name, N-(Methoxy-methylsulfanyfanylphosphoryl)acetamide (Fig. 1). Commercial names of $C_4H_{10}NO_3PS$ are Asataf 75% SP; Tremor 75 SP etc.

MCV (fL) = PCV (%) × 10 / RBC (10^6/L).

MCHC = Hemoglobin (g/dL) × 100 / PCV (%).

MCH (pg) = Hemoglobin (g/dL) × 10 RBC / (10^6/L).

Plasma glucose was determined using assay kits supplied by Human Diagnostics Worldwide according to [12]. Total protein (TP) content was determined according to the method by [13] and total lipid (TL) was determined by [14]. The activity levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined calorimetrically according to [15]. The nominal and measured concentrations were compared for significant difference using student t test using SPSS software the values considered significant at *p– value* < 0.05 and histology methods followed by Tamizhazhagan & Pugazhendy [16].

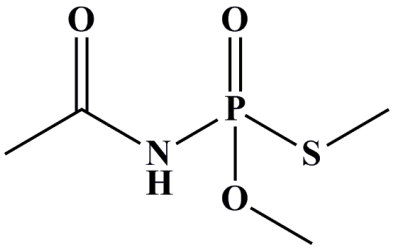


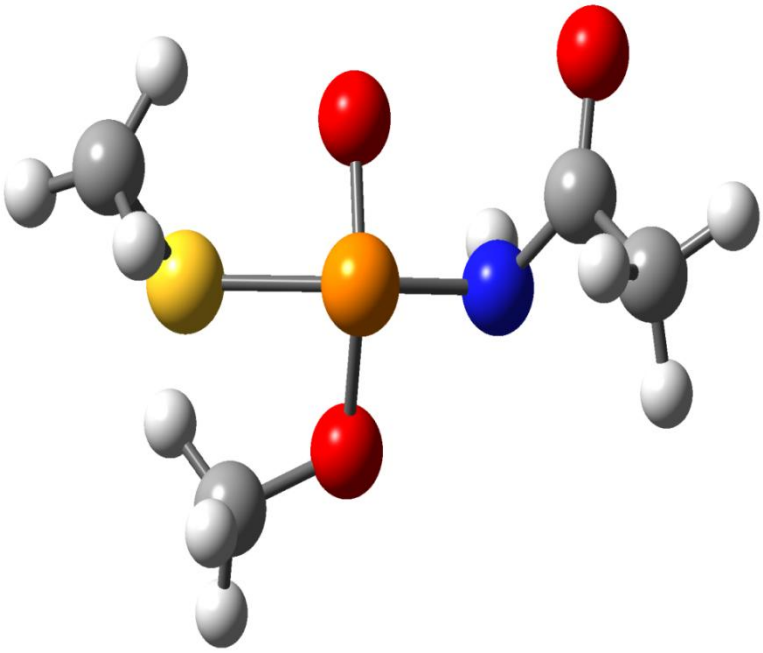
Fig. 1. *2D* structure of $C_4H_{10}NO_3PS$ and IUPAC name

3. RESULTS AND DISCUSSION

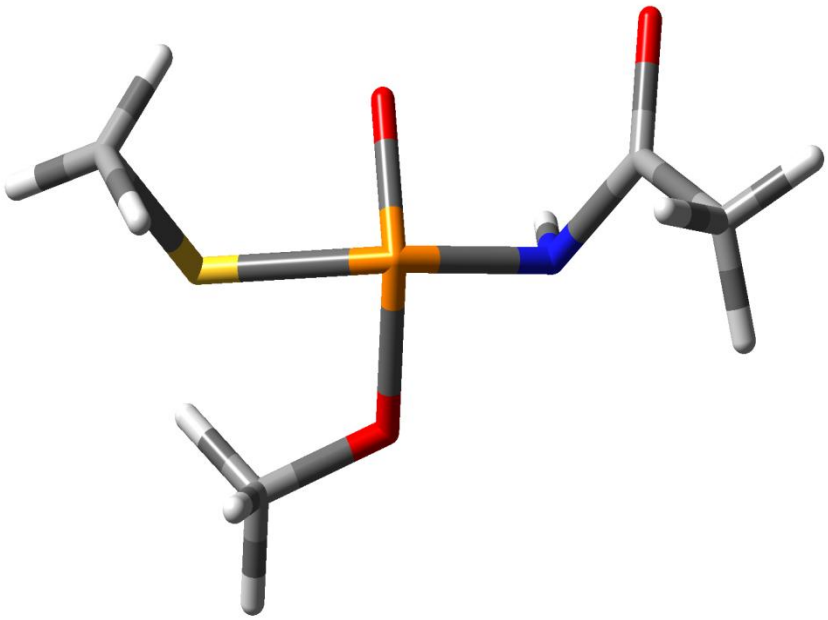
The result consequences of various blood parameters after toxicant presentation were given in Table 1, alongside the standard deviations and percent changes over control. Toxicants predominantly followed up on circulatory frameworks and significant effect on blood parameters. Thus our current investigation uncovered the impact of pesticide poisonousness on blood parameters. In studies, we have observed the significant changes in blood parameters after exposures of $C_4H_{10}NO_3PS$ during 1 day lethal, 1 day, 5 day and 10 day sublethal periods. The sublethal concentration of $C_4H_{10}NO_3PS$ is 910 mg/mL. In the present study the alterations in the hematological profiles were investigated in *C. punctata* after exposure of lethal and sublethal concentrations of $C_4H_{10}NO_3PS$ (It is showed *3D* normal and *3D* optimized structure in the Fig. 2a, b). So the pesticide had shown considerable impact on different blood parameters. The results of the present study were tabulated in Table 1. In the present study the RBC counts, WBC counts, HB and PCV levels were decreased significantly ($p<0.05$) when compared to control during lethal, 5 day and 10 day sublethal exposures of pesticide. MCH and MCHC levels and MCV the levels were increased compared to control. Glucose, TL, AST and ALT levels increased significantly after exposures of $C_4H_{10}NO_3PS$ but the Total Protein values were decreased in significantly.

Similar results were obtained during exposure of Azo dye [17]. The decrease in RBC and Hb content indicates acute anemia in exposed fingerlings. The anemia could be due to the destruction of RBC. The anemia may also be of hemolytic type. In the present investigation, hemolysis might have been one of the causes for reduction in Hb, RBC and PCV values. The fall in hematological parameters might be due to decreased rate of production or increased loss of destruction of RBC and another reason for decrease in RBC count due to damage to the hemopoietic tissue. Our results are in good agreement with earlier work that reported a decrease in RBC count, hemoglobin content and PCV of freshwater fish exposed to toxicants [18]. WBC count, and erythrocyte count and hemoglobin content decreased, while other indices like MCV, MCH and MCHC values were increased in all exposures in this study. PCV appears to be positively correlated with RBC counts, hence, a decrease in PCV levels was also observed. White blood cells in fish respond to various stressors including infections and chemical irritants. Thus, increasing or decreasing numbers of white blood cells are a normal reaction to a toxicant, which demonstrate the effect of immune system under toxic conditions [7]. The decreased number of WBC may be the result of bio concentration of the test pesticide in the kidney and liver. The erythrocyte constants MCV, MCH, and MCHC allow the determination of morphological anemia i.e., normocytic, macrocyte or microcytic anemia. The alterations in the hematological indices i.e. increase in MCV, MCH and MCHC in the present study may be due to a defense against the toxic effect of zinc metal ion and in turn due to decrease in RBCs, Hb and PCV and the disturbances occurred both in metabolic and haemopoietic activities in fish [19]. Percentage reduction in total erythrocytes noticed in the present study exhibited that *C. punctata* exposed to $C_4H_{10}NO_3PS$ became anemic, possibility due to hemodilution resulting from impaired osmoregulation across the gill epithelium. The effect of sublethal concentrations of propoxur has been reported by [20] with a significant decrease in hematocrit value and haemoglobin concentration in *Heteropneustes fossilis*

and decrease in hematocrit value and hemoglobin has been reported by [21] in *Anabas testudineus*. Our present results correlated with [22] results, they were studied same hematological parameters in *C. punctata* and *Barbes gonionotus* after diazinum 60 EC exposure. In their studies, they were noticed a significant decrement in RBC, Hb, Hct and MCHC values, but MVC value increased. In the same way our findings also some parameters were significantly decreased.



a) *3D* normal structure of $C_4H_{10}NO_3PS$



b) *3D* optimized structure of $C_4H_{10}NO_3PS$

Fig. 2. a) *3D* normal and b) *3D* optimized structure of $C_4H_{10}NO_3PS$

Table 1. Hematological changes after exposure of *C. punctata* to $C_4H_{10}NO_3PS$ for 1 day lethal, 1 day sublethal, 5 day sublethal and 10 day sublethal concentrations were analysed

Blood parameters	Control Mean ± SD	1 Day Lethal Mean ± SD	% Change	1 Day Sublethal Mean ± SD	% Change	5 Day Sublethal Mean ± SD	% Change	10 Day Sublethal Mean ± SD	% Change
RBC Count (10^6/mm^3)	2.63 ± 0.06	1.72 ± 0.03	-24.31	1.63 ± 0.06	-2.17	1.53+0.05	-7.31	1.32 ± 0.32	-27.31
WBC Count (10^3/mm^3)	13.10 ± 1.24	12.13+0.67	-22.56	15.31 ± 0.71	-4.51	13.22 ± 1.29	-5.26	13.28 ± 1.13	-7.58
HB (gm/100mL)	6.31 + 0.16	5.17 ± 0.31	-27.02	6.00 ± 0.42	-15.31	5.19 ± 0.16	-23.24	4.52 ± 0.24	-36.23
PCV (%)	31.13 ± 1.02	23.12+1.10	-25.34	29.10 ± 0.54	-6.14	26.42 ±1.77	-15.19	23.14 ± 1.45	-25.19
MCV (fL)	178.27 + 3.14	183.22 + 6.19	3.33	174.20 ± 2.17	0.52	180.17 + 4.19	1.06	182.24 + 5.15	2.78
MCH (pg)	37.69±2.39	53.81+1.14	23.83	43.14±2.11	0.42	55.44+1.63	13.86	54.81+1.16	20.78
MCHC (%)	30.18+3.25	35.17+2.28	16.33	32.41±1.10	7.38	33.20+1.44	14.00	34.18+0.64	13.25
Glucose (mg/L)	41.24+0.74	63.41+1.24	59.00	53.57±1.41	31.42	56.44+0.63	33.18	52.48+1.43	38.23
TP(g/100 mL)	3.16+0.48	1.32+0.41	-35.21	3.00 ±0.24	-5.20	2.18+0.24	-31.16	1.69+0.25	-44.29
TL(g/L)	12.11+0.49	15.17+1.52	36.13	14.15±0.51	27.49	15.17+0.72	34.00	14.55+0.98	30.19
AST (IU/L)	54.10+2.00	130.24+1.52	32.34	90.31±0.51	07.33	110.00+1.20	30.26	117.12+2.45	39.13
ALT (IU/L)	34.13+1.26	52.12+0.50	38.12	33.22±1.28	03.44	43.52+1.46	23.41	50.32+1.40	44.24

SD = Standard Deviation, fL = femtoliters, IU= International Unit, values are significant at p <0.05

The increase in MCH and MCHC in the present study clearly indicates the reduction in cellular blood iron, resulting in reduced oxygen carrying capacity of blood and eventually stimulating erythropoiesis MCH is a good indicator of RBC swelling. The significant increase in the MCHC values in the present study may be due to direct or feedback responses of structural damage to red blood cells membranes, resulting in hemolysis and impairment in hemoglobin synthesis and stress-related release of red blood cells from the spleen and hypoxia, induced by exposure to a toxicant [23]. The decrease in TEC over control was observed in all experimental periods of toxicant in 1 day, 5 days and 10 days. In 2006 [24] reported the reduction of hematological parameters in freshwater fish *Clarias batrachus*. In their reports they were noticed the decrement of RBC, WBC, PCV, MCH, MCV and its anemic conditions. These reports were supporting our present studies and their toxicants action in fish. Erythropoitin is a glycoprotein hormone that plays a crucial role in ensuring adequate supply of oxygen to tissues by regulating the production of erythrocytes [25]. Since the kidney of teleost was found to contain a higher level of the immune-reactive erythropoietin than other tissues, it is suggested that the kidney is the major erythropoietic, as well as erythropoietin - producing organ.

The main function of blood is transportation of oxygen and nutrients to cells as well as to remove cell metabolites from the body. When assessing the physiological effect of water toxicants on fish life, it becomes necessary to take into account the morphological changes occurring in the cells simply because changes in erythrocytes may cause an imbalance in the respiratory physiology of the fish. In the present study, it was observed that mature erythrocytes in the blood of *C. punctata* are elliptical in shape, the nucleus is also elliptical and centrally located (Fig. 3a). Erythrocytes exhibited different shapes due to the protrusion of cytoplasm in the form of projections (SS and LP) were seen in 24 hours lethal and sublethal exposures (Fig. 3b and 3c). Fish blood has two main types of cells, i.e., erythrocytes or RBC and Leucocytes or WBCs [26]. The frequency of occurrence is more in lethal exposure. Double lobopodial projections (DLP) and irregular of cells were found in 5 days sublethal exposures of $C_4H_{10}NO_3PS$ (Fig. 3d). Erythrocytes were found to be swollen and spherical (SS) in sublethal exposure for 10 days (Fig. 3e). The spherical erythrocytes may be referred to as 'spherocytes'. Number of such spherical erythrocytes increased significantly in lethal exposure. The swollen, oblong and erythrocytes (OS) were seen at 24 h sublethal exposure, which increased significantly upon exposure to 24 h lethal concentration of $C_4H_{10}NO_3PS$. The impact of $C_4H_{10}NO_3PS$ was so much deleterious at the lethal concentration than that of sublethal exposure. These RBCs and WBCs are developed from hemocytoblast precursor cells as well as mature cells after entering the blood stream. In fish blood erythrocytes are the most abundant cells as these contain hemoglobin which helps with the transport of oxygen from the gills to different body parts and shows pink colour when stained with Giemsa staining solution [27].

According to [28] significant decrease in RBC and PCV were observed in *Heteropneustes fossilis* when exposed to aldrin and fenvalerate pesticides. ESR was found for the fenevalerate treated groups, and MCH & MCHC values were found to decrease after pesticide treatment. Nath et al. [29] also reported a significant decrease in RBC, Hb, PVC and MCV in *H. fossilis* after their exposure to fenvalerate belonging to the pyrethroid group. These reports are strongly supported our present findings. Similar reports have been reported for several freshwater fishes [30]. Significant elevation or reductions in hematological values of fish exposed to different environmental toxicants have been reported by several workers as well as our present work. A reduction in leukocyte count (i.e., leucopenia) was observed in *C. punctata* after chronic exposure of monocrotophos by [31]. They observed leucopenia was due to increased activity of the pituitary internal stress axis. But in contrast, the number of leukocytes increased after 15 days exposure was observed. The increase in leukocyte count was correlated with an increase in antibody production that helps in survival and recovery of the fish exposed to a sublethal concentration of pesticide. The increase in WBC count can be correlated with an increase in antibody production, which helps in survival and recovery of the fish exposed to sublethal concentrations of pesticide [32]. The present findings also show hypersensitivity of leukocytes for pyraclostobin and these changes may be due to immunological reactions to produce antibodies to cope up with stress induced by $C_4H_{10}NO_3PS$.

A significant decrease in total leukocyte count (TLC) was observed in all the toxicants exposures. Maximum was recorded in the 10 days sublethal exposes and minimum increase was in 24 h lethal exposure. In the present study increase in total leukocyte count in the treated set was due to initiation of pathogenic condition, most likely in the form of irritation, injury to the cells and formation of tissue debris and occurrences of secondary infection in the fish body. This also helps in the removal of cellular debris of necrosis tissue under chemical stress. This also helps in the removal of cellular debris of necrosis tissue at a faster rate [33]. In the presence of foreign

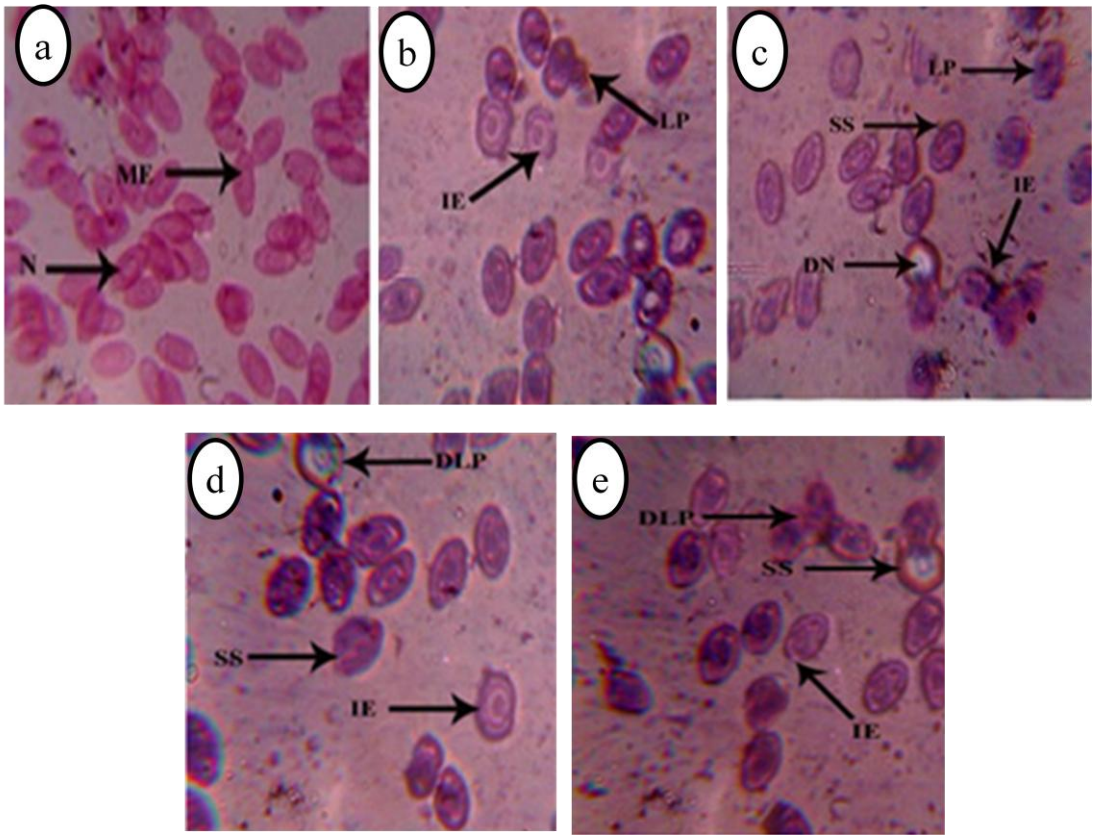


Fig. 3. Morphological changes of Erythrocytes in *Channa punctata*, exposed to lethal concentrations of Acephate for 24 h, sublethal concentrations of Acephate for 24 h, 5 Days and 10 Days (ME- Mature Erythrocytes, N- Nucleus, LP- Lobopodial Projections, SS- Swollen and spherical Erythrocytes, DN- degenerated nucleus, IE- Irregular Erythrocytes)

substances or under pathological conditions leucocytosis in fish may be the consequence of direct stimulation of immunological defense. A significant decrease in erythrocyte counts, hemoglobin, packed cell volume, mean corpuscular hemoglobin concentration and an increase of white blood corpuscles, mean corpuscular volume and mean corpuscular haemoglobin in the fish, *C. punctata* due to pollution from slaughter house wastes [34]. An increase in the TLC could be due to stimulated lymphopoiesis and/ or enhanced release of lymphocyte response might be due to the presence of toxic substances or may be associated with the pollutant induced tissue damage was also opined [35].

Haemoglobin percentage (Hb %) maximum reduction (27.08%) observed in 10 days sublethal exposures and minimum reduction was observed in 24 h sublethal exposure. Decrease in haemoglobin in experimental animals might be due to destruction of decrease in haemoglobin [21] in *Anabas testudineus*. Our present fingerlings are consistent with the above study that significant decrease in Hb% was observed in $C_4H_{10}NO_3PS$ 24 h and 5, 10 days both lethal and sublethal concentrations. On the other hand the increase of the hemoglobin [36] which could be due to the catalyzing actions of pesticides on the incorporation of body iron stored into haemoglobin. The Packed cell volume (PCV) appears to be positively correlated with erythrocytes count. Fall in the number of red blood cells followed by PCV confirms anemia in *Labeo rohita.* The decrease in PCV in fish may be due to the decrease of erythrocytes numbers, which in turn might be due to the $C_4H_{10}NO_3PS$ exposure. The decrease of PVC indicates anemia or aligoanemia condition in fish [37].

Alterations in MCV, MCH and MCHC also clearly indicate that the fish are under chemical stress, which leads to pathological conditions in the tissues. The change of variation over control exposures leads to a pathological condition in the tissues. Workers such as [38,39,40,41,42,8] were also studied the hemotological parameters in different fish under different chemical exposures, these are correlated the present observations. A hematological profile of an organism can provide important information about the

internal environment of the organism [43]. MCH values in *Oncorhynchus mykiss* exposed to cypermethrin. Das et al. [44] were studied the blood parameters such as TEC, TLC, Hb%, blood glucose, serum proteins and size & surface area of erythrocytes of Indian major carps *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala* exposed to two sublethal concentrations of acidic and alkaline water pH and found a decrease in the serum protein, Hb% and TEC levels but the blood glucose level and TLC were found to be elevated when compared with to control. Rainbow trout injected with a technical mixture of Delor 103 to evaluate the red blood cell indices (red blood cell count, hematocrit, haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin concentration) and some biochemical and enzyme parameters of the blood plasma (total protein, glucose) caused an increase in the red blood cell counts, hematocrit values, haemoglobin concentration [45]. The present study total serum proteins in the control fish was 3.16 g/ 100 ml and decrease in serum proteins over the control along with standard deviations represented in Table 1. Treated showed low values of serum protein levels than that of control. The highest percentage of reduction was observed in 10 days sublethal exposure and lowest percentage of reduction in fish *C. punctata* 24 h sublethal exposure. Das and Mukherjee, [46] reported total serum protein was decreased in fish *Labeo rohita* exposed to sublethal concentrations of quinalphos after 15, 30 and 45 days. The level of total protein was depleted, probably because of renal excretion (albuminuria) and impaired protein synthesis or was due to a liver disorder after the pesticide exposures. The experiments conducted [47] on fish, *C. punctata* exposed to sublethal concentrations carbamate fungicide-indofil on total serum proteins revealed that decrease in serum proteins was observed in all concentrations in different exposure period.

Proteins are indispensable constituents of the body and their metabolism is almost confined to the liver. Fall in serum protein level may be due to impaired function of kidney or due to reduced protein synthesis owing to liver cirrhosis [48,49]. Das and Mukherjee, [46] and Jenkins and Macpherson, [50] the reduction of protein content may be due to increased activity and decreased anabolic activity of protein as observed after toxicant exposures. In the present study the maximum elevation of blood glucose was in 10 days sublethal concentrations and minimum elevation was at 24 h sublethal exposure. Blood sugar levels are elevated in fish during acute exposure to a variety of compounds, including pesticides. The increase in blood sugar noticed in the present study could be attributed to differences in respiration and activity as pointed out [33]. The progressive accumulation of blood glucose reported in this investigation revealed that rohu exposed to sublethal concentrations of quinalphos became hyperglycemic. Omoregie [51] reported that tilapia showed marked hyperglycemic response to stressed environmental conditions as a result of incomplete metabolism of the blood sugar due to impaired osmoregulation.

Studies suggested that the blood indices of a fish species suffer changes related to variations in the aquatic environment [52]. Reduction of TEC and Hb% may be suggestive of an appreciable decline in the hematopoiesis leading to various types of anemia like poikilocythemic, microcytic and haemocytic anemia. Increase TLC is recorded probably due to thrombocytosis, lymphocytosis or leucopoiesis and/ or enhanced release of lymphocytes from the lymphoid tissues under the effect of toxic compounds. From the present study, $C_4H_{10}NO_3PS$ 24 h and 5 and 10 days exposures of lethal and sublethal concentrations can induce changes in the different blood parameters. Maximum effect was seen in 10 days exposure and minimum effects in 24 h exposures. The most severe attack of this pesticide leads to membrane disruptions and cytoplasmic blabbing. The erythrocyte membrane seems to be most affected depicting increased porosity. These changes might have resulted due to the disturbed lipid microenvironment of the membrane and more so, due to increased lipid peroxidation induced by the chemical, hence, resulting in increased membrane of the infected cell are manifested by the theological properties of the cells where they cannot traverse the microvasculature that leads to accelerated pitting and clearance within the spleen [53]. Studied the effect of monocrotophos on *Cyprinus carpio communis* and observed elliptical shape of the erythrocytes. Erythrocytes exhibited different lobopodial projections, discocytes, kerotocytes and bursiform red cells were observed in both lower and higher concentrations (0.15 ml/L for 35 days and 0.30 ml/L for 55 days). The swollen, oblong and shrieked erythrocytes were seen at lower concentration, which increased significantly upon exposure to higher concentration. The sublethal concentrations of quinalphos exposed to *Labeo rohita* in $C_4H_{10}NO_3PS$ toxicant caused erythrocytes enlargement, creation of cell wall, distortion, and hypertrophy of nucleus [46].

In the current examination decline in RBC may be come about because of the hindrance of RBC creation or because of the collection of effluents in the gill district causing harm in the structure of the gill bringing about hemolysis. A few creators have detailed the decrease of RBC in fish presented to toxins. The expansion in leukocyte tally noted is a reaction of creatures to adjust to the pressure

condition in the first place, and the ensuing decrease in leukocytes check demonstrates the debilitating of the invulnerable framework because of the more prominent pressure impact at higher fixations and time length. It is in concurrence with the report that the expansion in WBC in focused on creatures is a defensive reaction to push [54]. The immature red cells and hypochromia frequently observed at was corroborated with the study on the effects of pollution on *Gobius niger* [55]. In *Oreochromis niloticus* exposed to lead, the percentage of immature erythrocyte count and binuclear erythrocytes were found to increase [56]. Similarly the exposure of fish to ultra-violet radiation (320-400 nm) resulted micronuclei and binuclear erythrocytes were found in *Clarias gariepinus* [57]. The occurrence of vacuole in the cytoplasm of erythrocytes and changes in the nucleus was observed in *Gambusia affinis* for 0.1 ppm and 1.0 ppm Cu and Cd concentrations [58]. The gills in fishes are concerned with functions such as respiration and osmoregulation and are in close contact with the external environment.

4. CONCLUSION

Blood biochemical alteration occurs and many changes fish body. Finally, we conclude that lead acetate is highly toxic to fish, and impose life threatening effect on fish at both lethal and sublethal concentrations. The present study impact of lead acetate and ameliorative properties of *C. punctata* treated fish's aquatic ecosystems can affect the aquatic fauna in different ways. Long term exposure to these products causes countless abnormalities and reduces the life span of aquatic organisms. Altered hematological responses can be used as tools in bio-assessment to monitor eco-toxicological risks associated with pesticides such as lead acetate to various fish. It affected entire aquatic food chains spread generation to another generation.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ruby DS, Masood A, Fatmi A. Effect of aflatoxin contaminated feed on energy reserves of fish *Labeo rohita* (Hamilton). Current World Environment. 2014;9(3):1037.
2. Somaiah C, Kumar A, Mawrie D, Sharma A, Patil SD, Bhattacharyya J, Jaganathan BG. Collagen promotes higher adhesion, survival and proliferation of mesenchymal stem cells. PLoS One. 2015;10(12):e0145068.
3. Wilson R, Taylor E. The physiological responses of freshwater rainbow trout, *Oncorhynchus mykiss*, during acutely lethal copper exposure. Journal of Comparative Physiology B. 1993;163(1):38-47.
4. Das A, Mukherjee D. Soil application of insecticides influences microorganisms and plant nutrients. Applied Soil Ecology. 2000;14(1):55-62.
5. Kumar K. WIF India Workshop Report: Enhancing Women's Roles in Fisheries in India. YUVA Centre, Navi Mumbai, India: Report. 2010;1-3.
6. Ovie S, Ibiyo L, Babalola T, Eze S. The effects of varying levels of yeast (*Saccharomyces cerevisiae*) on the growth and body composition of *Heterobranchus longifilis* fingerlings. Zoologist (The). 2012;10.
7. Guedenon P, Edorh PA, Hounkpatin AS, Alimba CG, Ogunkanmi A, Nwokejiegbe EG, Bordeaux Cedex F. Haematological study of *Clarias gariepinus* exposed to chronic and subchronic doses of cadmium, mercury and combined cadmium and mercury. The Science of Nature. 2012;4(2):2-19.
8. Tilak K, Ranganayaki N, Pal K, De R, Saxena A, Nautiyal CS, Johri B. Diversity of plant growth and soil health supporting bacteria. Current Science. 2005;136-150.
9. Adhikari B, Howes T, Bhandari B, Troung V. Effect of addition of maltodextrin on drying kinetics and stickiness of sugar and acid-rich foods during convective drying: Experiments and modelling. Journal of Food Engineering. 2004;62(1):53-68.
10. Zhukov AE, Ustinov VM, Egorov AY, Kovsh AR, Tsatsul AF, Ledentsov NN, Alferov ZI. Negative characteristic temperature of InGaAs quantum dot injection laser. Japanese Journal of Applied Physics. 1997;36(6S):4216.
11. Blahova J, Modra H, Sevcikova M, Marsalek P, Zelnickova L, Skoric M, Svobodova Z. Evaluation of biochemical, haematological and histopathological responses and recovery ability of common carp (*Cyprinus carpio* L.)

after acute exposure to atrazine herbicide. BioMed Research International; 2014.

12. Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Annals of Clinical Biochemistry. 1969;6(1):24-27.
13. Henry RJ. Clinical chemistry, principles and techniques; 1964.
14. Placer ZA, Cushman LL, Johnson BC. Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems. Analytical Biochemistry. 1996;16(2):359-364.
15. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology. 1957;28(1):56-63.
16. Tamizhazhagan V, Pugazhendy K. Histological methods in life science. International Journal of Biomedical Research. 2017;5(6):68-71.
17. Barot J, Bahadur A. Toxic effect of azo dye (CI direct green 6) on blood parameters of freshwater fish *Labeo rohita* (Ham.). Journal of Cell and Tissue Research. 2014;14(2):4251.
18. Vutukuru S. Acute effects of hexavalent chromium on survival, oxygen consumption, hematological parameters and some biochemical profiles of the Indian major carp, *Labeo rohita*. International Journal of Environmental Research and Public Health. 2005;2(3):456-462.
19. Afaq S, Rana K. Toxicological effects of leather dyes on total leukocyte count of fresh water teleost, *Cirrhinus mrigala* (Ham). Biology and Medicine. 2009;1(2):134-138.
20. Singh SP, Gutierrez J, Molina A, Urrea C, Gepts P. Genetic diversity in cultivated common bean: II. Marker-based analysis of morphological and agronomic traits. Crop Science. 1991;31(1):23-29.
21. Bakthavathsalam R. Hematology of the fish Anabas testudineus exposed to lindane and carbofuran at submerged condition and on exposure to air. Environment and Ecology. 1991;9(1):124-127.
22. Rahaman SO, Harbor PC, Chernova O, Barnett GH, Vogelbaum MA, Haque SJ. Inhibition of constitutively active Stat3 suppresses proliferation and induces apoptosis in glioblastoma multiforme cells. Oncogene. 2002;21(55):8404.
23. Patel C, Burke JF, Patel H, Gupta P, Kowey PR, Antzelevitch C, Yan GX. Is there a significant transmural gradient in repolarization time in the intact heart? Response to Patel et al: Cellular Basis of the T Wave: A Century of Controversy. Circulation: Arrhythmia and Electrophysiology. 2009;2(1):80-88.
24. Patnaik L, Patra A. Haemoatopoietic alterations induced by carbaryl in *Clarias batrachus* (LINN). Journal of Applied Sciences and Environmental Management. 2006;10(3):5-7.
25. Batra S, Perelman N, Luck LR, Shimada H, Malik P. Pediatric tumor cells express erythropoietin and a functional erythropoietin receptor that promotes angiogenesis and tumor cell survival. Laboratory Investigation. 2003;83(10):1477.
26. Tamizhazhagan V, Pugazhendy K. The toxicity effect of monocrotophos 36% e. C on the haematology, *Labeo rohita* (Hamilton, 1882). International Journal of Current Pharmaceutical Research. 2015;7(4):92-95.
27. Tamizhazhagan V, Pugazhendy K, Sakthidasan V, Jayanthi C. The toxicity effect of Monocrotophos 36% EC on the histological changes in gill of *Labeo rohita*. International Journal of Innovative Research in Multidisciplinary Field. 2016;2(11):435-439.
28. Preeti SK, Panwar J. Mycorrhiza-its potential use for augmenting soil fertility and crop productivity. Physiology of Nutrition and Environmental Stresses on Crop Productivity. 2013;111.
29. Nath R, Raser K, Stafford D, Hajimohammadreza I, Posner A, Allen H, Wang K. Non-erythroid alpha-spectrin breakdown by calpain and interleukin 1 beta-converting-enzyme-like protease (s) in apoptotic cells: Contributory roles of both protease families in neuronal apoptosis. Biochemical Journal. 1996;319(3):683.
30. Rehulka J. Haematological analyses in rainbow trout *Oncorhynchus mykiss* affected by viral haemorrhagic septicaemia (VHS). Diseases of Aquatic Organisms. 2003;56(3):185-193.
31. Agrahari P, Singh V, Singh D. Toxicity of snail attractant pellets containing eugenol with respect to abiotic factors against the vector snail *Lymnaea acuminata*. Biological Agriculture & Horticulture. 2012;28(3):156-166.
32. Joshi SA, Tsai LW. Jacobian analysis of limited-DOF parallel manipulators. Paper presented at the ASME. International Design Engineering Technical Conferences and Computers and Information in Engineering Conference; 2002.
33. Tilak K, Veeraiah K, Butchiram M. Effect of phenol on haematological components of Indian major carps *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala*. Journal of

Environmental Biology. 2007;28(2):177-179.

34. Hymavathi V, Rao L. Effect of sublethal concentration of lead on the haematology and the biochemical constituence of *Channa punctata*. Bulletin of Pure and Applied Sciences. 2000;19:1-5.
35. Haniffa M. Haematological effects of textile mills effluents on freshwater fish, *Oreochromis mossambicus*. Environmental Research. 1990; 17:191.
36. Abidi R, Srivastava U. Effect of endosulfan on certain aspects of hematology of the fish, *Channa punctatus* (Bloch). Proceeding of the National Academy of Sciences. 1988;58(B):55-65.
37. Wepener V, Van Vuren J, Du Preez H. Effect of manganese and iron at a neutral and acidic pH on the hematology of the banded tilapia (*Tilapia sparrmanii*). Bulletin of Environmental Contamination and Toxicology. 1992;49(4):613-619.
38. Ahmed M, Sanders J, Nell W. New sorghum and millet cultivar introduction in Sub-Saharan Africa: Impacts and research agenda. Agricultural Systems. 2000;64(1):55-65.
39. Bhat BA, Bhat IA, Vishwakarma S, Verma A, Saxena G. A comparative study on the toxicity of a synthetic pesticide, dichlorvos and a neem based pesticide, neem-on to *Labeo rohita* (Hamilton). Current World Environment. 2012;7(1):157-161.
40. Gupta AK, Gupta M. Synthesis and surface engineering of iron oxide nanoparticles for biomedical applications. Biomaterials. 2005; 26(18):3995-4021.
41. Ololade I, Oginni O. Toxic stress and hematological effects of nickel on African catfish, *Clarias gariepinus*, fingerlings. Journal of Environmental Chemistry and Ecotoxicology. 2010;2(2):014-019.
42. Singh P, Tomer N, Kumar S, Sinha D. MHD oblique stagnation-point flow towards a stretching sheet with heat transfer. International Journal of Applied Mathematics and Mechanics. 2010;6(13):94-111.
43. Masopust D, Vezys V, Marzo AL, Lefrançois L. Preferential localization of effector memory cells in nonlymphoid tissue. Science. 2001;291(5512):2413-2417.
44. Das K, Lepoint G, Leroy Y, Bouquegneau JM. Marine mammals from the Southern North Sea: Feeding ecology data from δ13C and δ15N measurements. Marine Ecology Progress Series. 2003;263:287-298.
45. Řehulka J, Minařík B, Rehulková E. Red blood cell indices of rainbow trout *Oncorhynchus mykiss* (Walbaum) in aquaculture. Aquaculture Research. 2004; 35(6):529-546.
46. Das BK, Mukherjee SC. Toxicity of cypermethrin in *Labeo rohita* fingerlings: Biochemical, enzymatic and haematological consequences. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology. 2003;134(1):109-121.
47. Sharma A, Tyagi VV, Chen C, Buddhi D. Review on thermal energy storage with phase change materials and applications. Renewable and Sustainable Energy Reviews. 2009;13(2): 318-345.
48. Ravichandran KS. Signaling via Shc family adapter proteins. Oncogene. 2001;20(44): 6322.
49. Kumar S, Kumari K. Role of alkanols in micellar growth: A viscometric study. Journal of the American Oil Chemists' Society. 1995;72(7):817-821.
50. Jenkins D, Macpherson C. Transmission ecology of Echinococcus in wild-life in Australia and Africa. Parasitology. 2003; 127(S1):S63-S72.
51. Omoregie E. Changes in the haematology of the Nile tilapia, *Oreochromis niloticus* Trewavas under the effect of crude oil. Acta Hydrobiologica-Polish Academy of Sciences. 1998;40:287-292.
52. Das SK, Choi SU, Patel HE. Heat transfer in nanofluids—a review. Heat Transfer Engineering. 2006;27(10):3-19.
53. Sawhney A, Johal M. Erythrocyte alterations induced by malathion in *Channa punctatus* (Bloch). Bulletin of Environmental Contamination and Toxicology. 2000;64(3): 398-405.
54. Connors AF, Dawson NV, Desbiens NA, Fulkerson WJ, Goldman L, Knaus WA, Damiano A. A controlled trial to improve care for seriously iII hospitalized patients: The study to understand prognoses and preferences for outcomes and risks of treatments (SUPPORT). JAMA. 1995;274(20):1591-1598.
55. Katalay S, Parlak H. The effects of pollution on haematological parameters of black goby (*Gobius niger* L., 1758) in Foça and Aliağa Bays. Journal of Fisheries & Aquatic Sciences. 2004;21:113-117.
56. Al-Bairuty GA, Shaw BJ, Handy RD, Henry TB. Histopathological effects of waterborne copper nanoparticles and copper sulphate on

the organs of rainbow trout (*Oncorhynchus mykiss*). Aquatic Toxicology. 2013;126:104-115.

57. Kim D, Kim CH, Moon JI, Chung YG, Chang MY, Han BS, Lanza R. Generation of human induced pluripotent stem cells by direct delivery of reprogramming proteins. Cell Stem Cell. 2009;4(6):472-476.
58. Boran G, Karaçam H. Seasonal changes in proximate composition of some fish species from the Black Sea. Turkish Journal of Fisheries and Aquatic Sciences. 2011;11(1).