International Journal of Zoology and Applied Biosciences **ISSN: 2455-9571**
Volume 3, Issue 4, pp: 302-311, 2018
https://doi.org/10.5281/zenodo.1322891
http://www.ijzab.com

Research Article

HEMATOLOGICAL ALTERATIONS AFTER EXPOSURE PERIODS OF ACEPHATE IN FRESHWATER SNAKE HEADED FISH, *CHANNA PUNCTATA*

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Article History: Received 24th January 2018; Accepted 8th March 2018; Published 27th July 2018

ABSTRACT

The present study assesses the acute toxicity and behavioral alterations due to Acephate, an organophosphate pesticide on *Channa punctata*. The sublethal concentration of Acephate is 910 mg/L. In the present study, the alterations in the hematological profiles were investigated in *C. punctata* after exposure to lethal and sublethal exposures of Acephate. The values of different blood parameters after toxicant exposure and percent changes over control were presented. Toxicants mainly act on circulatory system and show major impact on blood parameters. Hence, our present study revealed the effect of pesticide toxicity on blood parameters. In our studies, we have observed the significant changes in blood parameters after exposed to 1 day lethal, 1 sublethal, 5 day sublethal and 10 day sublethal concentrations of Acephate. In the present study the RBC counts, WBC counts, Hb, and PCV levels were decreased significantly ($p < 0.05$) toxicant exposed fish when compared to control fish. The MCV, MCH and MCHC levels were increased in toxicant exposed fish when compared to control fish. Also the Glucose, TL, AST and ALT levels were increased significantly ($p < 0.05$) after exposure of Acephate but the TP values were decreased significantly.

Keywords: Acephate, *Channa punctata*, LC_{50}, Hematological parameters.

INTRODUCTION

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 (Ruby *et al.*, 2014; Somaiah *et al.*, 2015). 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. 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 (Priya *et al*., 2015).

Fish live in water and extremely close contact with their environment, and consequently they are very susceptible to any physicochemical changes which may be reflected in their blood parameters (Wilson and Taylor, 1993). 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 Das and Mukherjee (2000); Kumar (2010); Ovie *et al.* (2012) 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. 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 (Shalaka Sadekarpawar and Parikh, 2015).

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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 (Guedenon *et al.*, 2012). 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. 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 (Tilak *et al.*, 2005).

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 (Adhikari *et al.*, 2004; Zhukov *et al.*, 1997). 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 (Shalaka Sadekarpawar and Parikh, 2015) 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 (Blahova *et al.*, 2014). 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.

Along with alterations in the hematological profile the fishes also exhibit alterations in the metabolism and biochemical processes (Shalaka Sadekarpawar and Parikh, 2015). For example, several studies indicate that after exposure to a toxicant, fish may exhibit an increase or decrease in levels of plasma glucose, serum protein, creatinine and urea. However the exact changes can vary depending on the toxicant type, species of fish, water quality and length of exposure (Priya *et al.*, 2015). In this study, the effect of the Acephate on the hematological and biochemical profile of freshwater teleost fish, *Channa punctata* was studied. Such a study is vital as it not only assesses the health of fish subjected to changing environmental conditions but also for the deteriorating water quality.

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.

Pesticide: The commercial grade formulations of Acephate 75 % SP an organophosphate pesticide is used as a toxicant in the present experiment. Commercial names of Acephate are Asataf 75% SP; Tremor 75 SP etc. and its molecular formula is $C_4H_{10}NO_3PS$.

A common stock solution of Acephate was prepared by dissolving 1 gram (1000 mg) of pesticide in 100 mL of acetone and the required quantity of Acephate was drawn from the stock solution to maintain the standard concentration of 1 mg/L in the container. The acclimatized fish were placed into separate container containing dechlorinated and aerated water. The Pilot experiments were conducted to derive the LC_{50} values of 24 h, 48 h, 72 h and 96 h for Acephate. The sublethal concentration of Acephate is 910 mg/mL which was reported in our previous paper. Then the fish were exposed to pesticide for 24 h, 5 and 10 days to find hematological changes. During the whole experiment, a control group was maintained with acetone for comparison.

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.

The WBC lying inside the four large squares was counted under high power (40X) of light microscope. The following formula was used to calculate the number of WBC per mm^3 (µL) of the blood sample:

WBC = (N × dilution)/ area counted × depth of fluid.

Some blood dropped on glass slides prepared and were done on blood films stained with Giemsa. Replicate counts were made for each blood sample. Hemoglobin concentration was determined by the cyanmethemoglobin procedure (Boehringer Mannheim Kit, 124729). All colorimetric determinations were performed using a spectrophotometer (Perkin-E lmer Coleman) at 415 nm. To determine PCV/ HCT, duplicate fresh blood samples were collected into heparinized micro hematocrit tubes sealed with plasticine at one end and centrifuged for 5 min (H ermle Z 383 K) at 3000 rpm. The mean values of PCV (%) were measured with a microhematocrit reader. Hematological indices were calculated from the equations given by Ovie *et al*. (2012).

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 (Trinder, 1969). Total protein (TP) content was determined according to the method by Henry, 1964 and total lipid (TL) was determined by Placer *et al*. (1966). The activity levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined calorimetrically according to Reitman and Frankel (1957).

The nominal and measured concentrations were compared for significant difference using student t test using SPSS software the values considered significant at $p - value < 0.05$.

RESULTS

The sublethal concentration of Acephate 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 Acephate. So the pesticide had shown considerable impact on different blood parameters. The outcome results of different blood parameters after toxicant exposure were given in Table 1, along with the standard deviations and percent changes over control. Toxicants mainly acted on circulatory systems and major impact on blood parameters. Hence our present study revealed the effect of pesticide toxicity on blood parameters. In our studies we have observed the significant changes in blood parameters after exposures of Acephate during 1 day lethal, 1 day, 5 day and 10 day sublethal periods. 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 Acephate but the Total Protein values were decreased in significantly.

Table 1. Hematological changes after exposure of *Channa punctata* to Acephate for 1 day lethal, 1 day sublethal, 5 day sublethal and 10 day sublethal concentrations.

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)	1.73 ± 0.06	1.32 ± 0.03	-23.32	1.68 ± 0.06	-2.12	1.56+0.05	-9.31	1.36 ± 0.32	-21.31
WBC Count (10^3/mm^3)	16.41 ± 1.24	12.10+0.67	-26.56	15.61 ± 0.71	-4.51	14.22 ± 1.29	-5.26	13.28 ± 1.13	-7.58
HB (gm/100mL)	7.11 + 0.16	5.17 ± 0.31	-27.02	6.00 ± 0.42	-15.31	5.19 ± 0.16	-27.24	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 + 4.14	184.22 + 6.19	3.33	179.20 ± 2.17	0.52	180.17 + 4.19	1.06	183.24 + 5.15	2.78
MCH (pg)	48.69±2.39	59.81+1.14	22.83	49.14±2.11	0.92	55.44+1.63	13.86	58.81+1.16	20.78
MCHC (%)	30.18+4.25	35.17+2.28	16.53	32.41±1.10	7.38	33.20+1.44	10.00	34.18+0.64	13.25
Glucose (mg/L)	42.24+0.74	67.41+1.24	59.00	55.57±1.41	31.42	56.44+0.63	33.18	58.48+1.43	38.23
TP(g/100 mL)	3.16+0.48	1.72+0.41	-45.21	3.00 ±0.24	-5.20	2.18+0.24	-31.16	1.69+0.25	-46.29
TL(g/L)	11.11+0.49	15.17+1.52	36.13	14.15±0.51	27.19	15.17+0.72	36.00	14.55+0.98	30.19
AST (IU/L)	84.10+2.00	120.24+1.52	42.34	90.31±0.51	07.33	110.00+1.20	30.26	117.12+2.45	39.13
ALT (IU/L)	35.13+1.26	52.12+0.50	48.12	36.22±1.28	03.14	43.52+1.56	23.21	50.22+1.40	42.24

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

Morphological changes

Fish blood has two main types of cells, i.e., erythrocytes or RBC and Leucocytes or WBCs. 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.

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 (Figure 1). 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 (Figure 2 and 3).

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 Acephate (Figure 4). Erythrocytes were found to be swollen and spherical (SS) in sublethal exposure for 10 days (Figure 5). 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 Acephate. The impact of Acephate was so much deleterious at the lethal concentration than that of sublethal exposure.

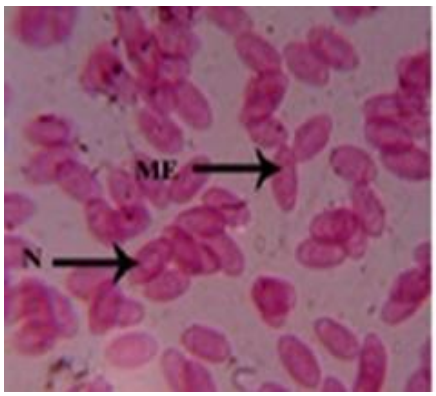

Figure 1. Coated

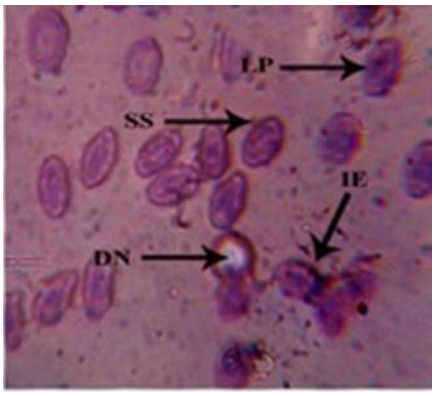

Figure 2. 1 Day Lethal

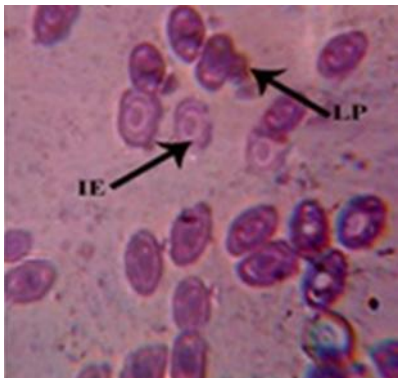

Figure 3. 1 Day Sublethal

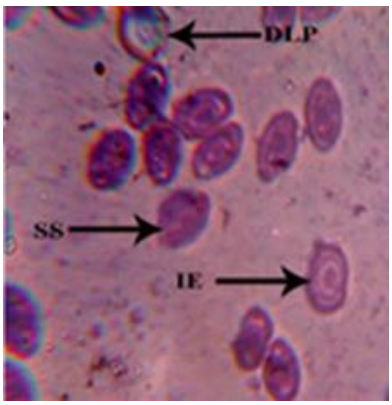

Figure 4. 5 Day Sublethal

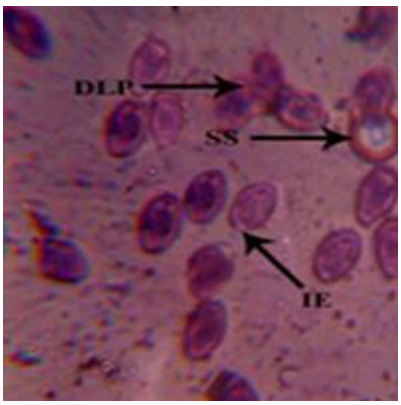

Figure 5. 10 Day Sublethal

Figure 1-5. 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).

DISCUSSION

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 (Vutukuru, 2005). 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. Similar results were obtained during exposure of Azo dye (Barot and Bahadur, 2014). 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. 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 (Guedenon *et al.*, 2012).

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 (Afaq and Rana, 2009).

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(Patel *et al.*, 2009).

Percentage reduction in total erythrocytes noticed in the present study exhibited that *C. punctata* exposed to Acephate 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 Singh *et al.*(1991) with a significant decrease in hematocrit value and haemoglobin concentration in *Heteropneustes fossilis* and decrease in hematocrit value and hemoglobin has been reported by Bakthavathsalam, (1991) in *Anabas testudineus*. Our present results correlated with (Rahaman *et al.*, 2002) 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.

The decrease in TEC over control was observed in all experimental periods of toxicant in 1 day, 5 days and 10 days. In 2006 (Patnaik and Patra, 2006) 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. (Batra *et al.*, 2003). 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.

According to Preeti and Panwar (2013) 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.*, 1996) 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 (Rehulka, 2003). 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 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 (Tilak *et al.*, 2007). In the presence of foreign 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 was reported by

Hymavathi and Rao (2000). 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 by Haniffa (1990).

A reduction in leukocyte count (i.e., leucopenia) was observed in *C. punctata* after chronic exposure of monocrotophos by Agrahari *et al*. (2012). 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 (Joshi and Tsai, 2002). 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 (Joshi and Tsai, 2002). 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 Acephate.

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 has been reported by Bakthavathsalam, (1991) in *Anabas testudineus*. Our present fingerlings are consistent with the above study that significant decrease in Hb% was observed in Acephate 24 h and 5, 10 days both lethal and sublethal concentrations.

On the other hand the increase of the hemoglobin was reported by Abidi and Srivastava (1988) 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 Acephate exposure. The decrease of PVC indicates anemia or aligoanemia condition in fish (Wepener *et al.*, 1992)

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 (Ahmed *et al*., 2000; Bhat *et al.*, 2012; Gupta and Gupta, 2005; Ololade and Oginni, 2010; Singh *et al*., 2010; Tilak *et al.*, 2005) 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 (Masopust *et al*., 2001). MCH values in *Oncorhynchus mykiss* exposed to cypermethrin. Das *et al.* (2003) 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 by Rehulka *et al*. (2004).

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 *et al*. (2003) 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 by Sharma *et al*. (2009) 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 (Ravichandran, 2001) and (Kumar and Kumari, 1995). Das and Mukherjee, (2003) and (Jenkins and Macpherson, 2003) 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 by Tilak *et al*. (2007). The progressive accumulation of blood glucose reported in this investigation revealed that rohu exposed to sublethal concentrations of quinalphos became hyperglycemic. (Omoregie, 1998) 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 (Das *et al.*, 2006) 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, Acephate 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 (Sawhney and Johal, 2000). Studied the effect of monocrotophos on *Cyprinus carpio communis* and observed elliptical shape of the erythrocytes. Erythrocytes exhibited different lobopodial projections, discocytes, kerotocytes and fusiform 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 Acephate toxicant caused erythrocytes enlargement, creation of cell wall, distortion, and hypertrophy of nucleus according to (Das and Mukherjee, 2000).

A significant reduction in haemoglobin content and erythrocyte count in the blood of a freshwater fish, *Sarotherodon mossambicus* on exposure to an organophosphate and a carbamate pesticide(Ramasamy *et al*., 2007). In the present study the surface area reduction of erythrocytes noticed it is suggestive of hypoxic effect prevailing over the body tissues has been reported by Das and Teng, (1998) in rohu fingerlings due to the effect of quinalphos on gill tissue. Sawhney and Johal (2000) studied erythrocytes alterations induced by malathion in *Channa punctata* and found erythrocytes were swollen when the fish exposed to 0.05mg/ L for 5 days. The number of such spherical erythrocytes increased significantly upon exposure for 15.to 30 days. Present study increase in the concentration of pesticide for the exposure period of Acephate was registered an increase in the number of such cells. Increase anisocytosis was a predominant feature, whereas a significant variation in shape and size of cells was noticed. Lobopodial projections were seen upon exposure to different levels of pesticides for different exposure periods.

Acephate caused clubbing of irregularly shaped erythrocytes as chains and cytoplasmic content was also found to ooze out resulting in formation of cremated cells with numerous projections. The chronic exposure caused cytoplasmic blending and ultimately the oozing out of the cytoplasmic content in a thread - like from called acanthocytes. Vacuolation in the cytoplasmic zone of the erythrocytes were also observed and the extent of vacuolization was found to be directly proportional to the pesticide dose and exposure period. Jayashree *et al.* (2015) also reported the effects of an organophosphate pesticide Acephate (75% SP) on Indian Major Carp *Labeo rohita* on the basis of the results of sub lethal toxicity tests, biochemical estimations and hematological indices. Experimental carp was exposed to the pesticide Acephate (75% SP) in the concentration of 0.0004 ml/lit in 24 h sublethal toxicity test by the static bioassay method. In hematological profile the experimental group of carp showed a significant ($P < 0.01$) increases the number of erythrocytes MCH, MCV and MCHC; decrease in the segmented neutrophils, granulocytes, and leucocyte count. The exposure of *Labeo rohita* to 10 g/lit Acephate caused significant shifts in hematological and biochemical profile (Jayashree *et al*., 2015)

In the present investigation decrease in RBC might be resulted from the inhibition of RBC production or due to the accumulation of effluents in the gill region causing damage in the structure of the gill resulting in hemolysis. Several authors have reported the reduction of RBC in fish exposed to pollutants. The increase in leukocyte count noted is a response of animals to adapt to the stress condition in the beginning, and the subsequent decline in leukocytes count indicates the weakening of the immune system due to the greater stress effect at higher concentrations and time duration. It is in agreement with the report that the increase in WBC in stressed animals is a protective response to stress (Connors *et al*., 1995).

The immature red cells and hypochromia frequently observed at was corroborated with the study on the effects of pollution on *Gobiusniger* (Katalay & Parlak, 2004). In *Oreochromis niloticus* exposed to lead, the percentage of immature erythrocyte count and binuclear erythrocytes were found to increase (Al-Bairuty et al., 2013). Similarly the exposure of fish to ultra-violet radiation (320-400nm) resulted micronuclei and binuclear erythrocytes were found in *Clarias gariepinus* (Kim *et al.*, 2009). 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 (Boran and Karacam, 2011). The gills in fishes are concerned with functions such as respiration and osmoregulation and are in close contact with the external environment.

CONCLUSION

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. 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. 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.

ACKNOWLEDGEMENT

The authors are thankful to the Co-ordinator, Department of Zoology and Aquaculture, Acharya Nagarjuna University for providing necessary laboratory facilities.

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