INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
19–0018/2019/22–4–703–709
DOI: 10.17957/IJAB/15.1119
http://www.fspublishers.org

Full Length Article

Hematological, Biochemical and Histopathological Alterations in Common Carp during Acute Toxicity of Endosulfan

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Abstracts

The organochlorine pesticide endosulfan has been banned in many countries due to their dangerous effects on the health of many non-target organisms, but they are still used in agriculture to increase crops yield. This study aimed to investigate the effects of the sub-lethal concentration of endosulfan on hematology, histopathology and biochemical parameters of common carp (*Cyprinus carpio* L.). A total of 30 common carp were selected and divided into five groups (E0, E1, E2, E3 & E4), each group contained six fishes. The common carp were exposed to sublethal concentration of endosulfan for 96 h at different concentration 0, 1, 3, 5 and 7 ppb, respectively. The hematological results showed that platelets count, leukocytes, MCH and MCV was significantly increased ($P < 0.05$) during acute toxicity of endosulfan on the other hand erythrocytes, monocytes, hemoglobin, and MCHC values significantly decreased ($P < 0.05$) compared to control. The biochemicals results showed a significant reduction ($P < 0.05$) in total protein, urea, albumin and globulin levels as the concentration of endosulfan increased, while significant increased ($P < 0.05$) was recorded in glucose level. In the present research, the significant histopathological alteration was recorded in gills, liver, brain, and intestinal tissue of common carp, exposed to different concentration of endosulfan. The results concluded that endosulfan has a strong influence on hematological, biochemical and histopathological parameters of common carp during acute toxicity.

Keywords: Biochemical; Common carp; Pesticide; Endosulfan; Histopathological; Hematological

Introduction

Pesticides are used worldwide in various agricultural fields to control the population of pest and insects (Yonar*et al*., 2012). Use of pesticides develops various behavioral changes in individuals. Various types of physiological and morphological alterations in individuals can cause due to the use of pesticides in the ecosystem. A broad spectrum of biological effects can cause by pesticides as they are environmental toxins, they have harmful effects not only on organisms but on humans as well (Jamil *et al.*, 2007). Endosulfan belong to the cyclodiene group 2, 3, 4 benzo dioxathiepen-3oxide) (6, 7, 8, 9, 10, 10-hexachloro-1, 5, 5a, 6, 9, 9a hexa hydro-6, 9-methane). Its half-life in water is about more than 14 days while in the soil around 60 to 800 days (Sarma *et al.*, 2012). In soy, cotton and coffee crops endosulfan is used for the control of insect and mites it is also used in tea, maize and fruits crops (Miglioranza *et al*., 2002). For the protection of wood from termites, endosulfan is also used. Endosulfan is used extensively in Pakistan for pest control in vegetables, cereal crops fruit crop and oilseed (Tariq *et al*., 2007). For mosquitoes and tsetse fly control programs endosulfan is also used (Guo *et al*., 2008). If humans are eating the food poisoned with endosulfan then they are probably exposed to it, low level exposure, by nose, direct skin contact with soil which is contaminated, whole-body inhalation exposure in the farms during its pertinence or endosulfan having leftover on the smoking cigarettes made from tobacco (Uboh *et al.*, 2011). Pesticides seriously affect aquatic biota most probably fish when it reaches the nearby water bodies along with agricultural runoff (Murthy *et al*., 2013). Endosulfan can interrupt reproduction in fish, and it affects the growth of fish (Balasubramani and Pandian, 2008). Demolition of the testis can also cause by the harmful effects of endosulfan (Dutta *et al*., 2006), leads to loss of oxygen (Ballesteros *et al*., 2009) also cause alterations in thyroid hormones (Coimbra *et al*., 2005). In addition to this, the development of fish can also be affected by endosulfan (Willey and Krone, 2001). Endosulfan can lead to reducing the feeding mode in fish (Giusi *et al*., 2005); the reproductive physiology is also altering by endosulfan (Gormley and Teather, 2003). The present study was conducted to find the sublethal effect of the most extensively used pesticide endosulfan in Pakistan on

To cite this paper: Khan, M.F., S. Tabassum, H. Sadique, M. Sajid, S. Ghayyur, K.D. Badshah, N. Khan and I. Ullah, 2019. Hematological, biochemical and histopathological alterations in common carp during acute toxicity of endosulfan. *Intl. J. Agric. Biol*., 22: 703–709

hematological, histopathological parameters on serum biochemical parameters of common carp (*Cyprinus carpio*).

Materials and Methods

Fish Collection

Total thirty Common carp (*C. carpio*) 5–6 cm in size were captured with the help of nets from Tarbela lake, Haripur, KP and then shifted to the laboratory of Department of Zoology, Govt. Postgraduate College Haripur. Careful netting and handling were done in order to minimize the stress on fish. The time taken from catching the fish and moving to the laboratory was approximately 3 h. After some time, the fish were examined for the presence of an external parasite. For a few seconds, the fish were placed in water containing 0.2% K_2MnO_4 then the washed with tap water.

Acclimatization

The fish were shifted to aquariums to adjust with laboratory conditions for a week before the experiment. They were kept in 60 L oxygenated glass aquarium having dechlorinated normal water having pH 7.6–7.8, the temperature was 20–23°C and dissolved oxygen 100% saturation. Pelleted commercial fed was given to fish daily during the acclimatization period. To maintain the proper hygiene of fish 70% of the water was removed after every 24 h.

Chemicals

The commercial formulation of organochlorine insecticide Endosulfan (35 EC) was used.

Experimental Design

Test solutions of four different concentrations 0,1, 3, 5 and 7 ppb were prepared of endosulfan from a commercial formulation consisting of 35% active ingredient to study the sub-lethal effects of a hazardous pesticide such as endosulfan on *C. carpio*. Five glass aquaria were selected for the fishes on which the experiment was done. The aquaria were having 60 L water capacity and filled with 30 L of water per tank. Six fishes were kept in each aquarium randomly. These were treated with the five-various concentration of endosulfan labeled such as E1, E2, E3, and E4 for experimental groups, while one aquarium E0 had no concentration of Endosulfan served as a control group. Water was freshened in each aquarium daily, and the chemical solution was also refreshed after every 24 h throughout the experiment.

Collection of a Blood Sample

The fishes were removed and anesthetized in clove oil after 96 h. About 2 mL blood samples were collected from the caudal vein of fish by the help of a heparin-coated disposable sterile syringe. Then shifted at once to EDTA tube consisting of a fine layer of heparin as an anticoagulant.

Determination of Hematological Parameters

The fish blood samples that were collected from both control, as well as intoxicated fish of experimental groups, were used for of neutrophils determination, total RBCs, monocytes, WBC count, platelets count, lymphocytes, HCT, and hemoglobin content determination with the help of automated hematological analyzer (Sys-Max Kx-121). The hematological parameters were examined of all six collected fish. Erythrocyte indices like MCV, MCH, and MCHC were also calculated according to standard formulas (Ghayyur *et al*., 2019).

Biochemical Analysis

The collected blood serum of both experimental and control groups of fish were used for the determination of metabolites such as total protein, Urea, Albumin, Cholesterol, Glucose, Globulin, Bilirubin and Triglycerides, Enzyme Amylase and Electrolytes such as Phosphorus and Calcium, with the help of Hitachi Model 917 Multichannel Analyzer (Rosch Germany).

Removal of Selected Biomarker Organs

The fish were sacrificed for selected biomarker organs, such as brain, heart, liver, intestine and gills. Each organ was placed separately and carefully in plastic containers in absolute alcohol.

Histopathological Analysis

The fish that was previously used in hematological parameters were also used for histopathological analysis. Microtomy procedure was used to study the histo-pathological change in intestinal tissues (Khan *et al*., 2018).

Statistical Analysis

Data were analyzed using SPSS software (Version 24). The comparison was made among all of the four experimental assemblies using one-way ANOVA. It was then followed by a comparison of hematological variables using independent *t*-test between control and treated groups. Variables were stated as means and standard deviations. $P < 0.05$ was considered as statistically significant.

Results

The present study was conducted to find out the toxic effects of endosulfan on hematological, histopathological and biochemicals parameters of common carp during acute toxicity.

Effects of Endosulfan on Hematological Parameters

Common carp were exposed to sublethal concentration of endosulfan for 96 h at different concentration 0, 1, 3, 5 and 7 ppb respectively. Hematological results showed that platelets count, leukocytes, MCH and MCV was significantly increased ($P < 0.05$) during acute toxicity of endosulfan with an increase in the concentration of pesticide. While erythrocytes, Monocytes, Hemoglobin, and MCHC values significantly decreased ($P < 0.05$) compared to control with an increased concentration of pesticide (Table 1).

Effects of Endosulfan on Biochemical Parameters

The given table shows the summary of the mean values of different biochemical parameters of both control and treated groups, expressed as the mean significant difference ($P < 0.05$) and significant intergroup difference. It was noted that amylase enzyme, Glucose (mg/dL), Bilirubin (mg/dL) & Cholesterol (mg/dL) showed increase in the values while Triglycerides (mg/dL), Albumin (g/dL), Urea (mg/dL), Globulin (g/dL), Calcium (mg/dL), Phosphorus (mg/dL) and total proteins (g/dL) were decreased with increased concentration of endosulfan respectively (Table 2).

Histopathological Changes in Gills

The present study was conducted to check out the effects of endosulfan on the histology of *C. Carpio* during acute toxicity. Different types of alterations were observed in the different organ as the concentration of endosulfan increased.

After the experiment of 96 h, the gills of the control group were observed under a light microscope. It showed that the gills of *C. carpio* have a thin epithelial covering, primary and secondary gill lamellae having a central cartilaginous core and the gill filament have a healthy structure. There is a thick lining of the stratified epithelium at the mid of primary and secondary gill lamellae consisting of chloride and mucous cells.

After the exposure of endosulfan on *C. carpio* for 96 h, results revealed different types of histopathological alterations in gills tissues compared to control group. When fish were exposed to a 1 ppb concentration of endosulfan in experimental group I, it showed disruption of cartilage & epithelial lifting. The shortening and division of secondary gills lamellae in the gills of *C. carpio* also seemed. When the fish of experimental groups II, III, IV were exposed to the sublethal concentration of endosulfan 0, 1, 3, 5 and 7 ppb the alterations in gills tissues became more prominent such as the fusion, shortening, and disruption of cartilaginous core of the fish gills, epithelial lifting, division of cartilaginous core, atrophy, lamellar curling and shortening secondary gills as shown in Fig. 1.

Histopathological Alterations in Liver

The fish of control group's liver tissue section of *Cyprinus carpio* had typical structures of liver cells with foamy cytoplasm when observed under light microscope Hepatocyte is around polygonal cell body containing a nucleus, an evident spherical nucleolus, and nucleus.

The treated group fish exposed to 1 ppb concentration of endosulfan showed that the hepatocytes have mild Pyknosis, Vacuolations, and lamellar in-fusion. While experimental group-II exposed to 3 ppb concentration of endosulfan, a dissolution of the cell membrane at different spots, Lymphocyte Infiltration (Li) & Pyknosis appeared. The third experimental group exposed to 5 ppb of endosulfan showed several alterations in tissues of the fish liver such as Vacuolations, Necrosis, Pyknosis & Lymphocyte Infiltration. The results of experimental group-IV, when exposed to 7 ppb concentration of endosulfan showed an infusion of the lamella, vacuolations, blood clogging, pyknosis and necrosis as shown in Fig. 2.

Histopathological Alterations in Brain

The brain section of control group *Cyprinus carpio* under the light microscope showed standard tissue alterations. When fish of group I exposed to 1 ppb concentration of endosulfan mild necrosis was observed, group II exposed to 3 ppb of endosulfan showed blood congestion, necrosis, and mild pyknosis. While the fish of group III and IV were exposed to 5 and 7 ppb of endosulfan alterations in the brain tissues were noticed such as congestion of blood and necrosis as in Fig. 3.

Histopathological Changes in Intestine

The intestine section of control group *C. carpio* under the light microscope showed standard tissue architecture of villi. The typical architecture of villi consists of lamina properia, muscularis mucosa, serous membrane, stratum compactum, and mucosal epithelium.

The group I showed detachment of villi, atrophy, and hemorrhages when exposed to 1 ppb endosulfan. Group II, when exposed to 3 ppb to endosulfan resulted in the detachment of villi, hemorrhages, detachment of villi & necrosis. The exposure of experimental group III and IV to 5 and 7 ppb of endosulfan revealed different histopathological changes such as detachment of villi, necrosis, and Hemorrhages as shown in Fig. 4.

Discussion

The current study aimed to investigate the sub-lethal effects caused by endosulfan on the hematological, histopathological and biochemical parameter of common carp (*C. carpio*) at 96 h. The results showed that exposure of *C. carpio* to 0, 1, 3, 5 and 7 ppb concentrations of endosulfan causes several alterations in hematological parameters *i.e.* WBC, MCV, MCH, and platelets increased significantly ($P < 0.05$) while hematocrit (HCT), erythrocyte count (RBCs), hemoglobin (Hb) level, mean corpuscular

Table 1: Hematological parameters of control and experimental groups of fish exposed to a sub-lethal concentration of endosulfan

Hematological Parameters	The concentration of endosulfan				
	0 ppb	1 ppb	3 ppb	5 ppb	7 ppb
Total WBC (103/mm^3)	68.64 ± 0.08	72.65 ± 0.10$^{\Delta}$	75.47 ± 0.10$^{\Delta}$	79.3740 ± 0.54$^{**\Delta}$	93.118 ± 0.83$^{**\Delta}$
Neutrophils%	17.40 ± 10.47	22.80 ± 3.96$^{\Delta}$	27.60 ± 1.14$^{\Delta}$	31.80 ± 2.55$^{\Delta}$	37.80 ± 1.64$^{\Delta}$
Lymphocytes%	63.00 ± 11.00	65.20 ± 1.78$^{\Delta}$	69.40 ± 1.81$^{\Delta}$	75.20 ± 2.38$^{\Delta}$	79.60 ± 1.81$^{\Delta}$
Monocytes%	7.60 ± 3.28	8.40 ± 1.14$^{\Delta}$	10.40 ± 0.54$^{\Delta}$	13.20 ± 1.30$^{\Delta}$	15.40 ±0.54$^{\Delta}$
RBCs (106 /ml)	4.42± 0.032	3.51 ± 0.05$^{\Delta}$	2.274 ± 0.30$^{**\Delta}$	1.550 ± 0.07$^{**\Delta}$	0.836 ± 0.041$^{**\Delta}$
Hb (g/dL)	11.66 ± 0.35	10.22 ± 0.13$^{**\Delta}$	9.4200 ± 0.23$^{\Delta}$	07.00 ± 0.45$^{**\Delta}$	04.44± 0.296$^{**\Delta}$
HCT (%)	17.26 ± 0.23	16.12 ± 0.19$^{**\Delta}$	14.52± 0.27$^{**\Delta}$	11.40 ± 0.26$^{**\Delta}$	08.36 ± 0.240$^{**\Delta}$
MCV (fL)	45.14 ± 13.74	53.60 ± 19.18$^{\Delta}$	59.02 ± 7.61$^{**\Delta}$	66.960 ± 3.14$^{**\Delta}$	71.12 ± 9.95$^{\Delta}$
MCH (pg)	23.78 ± 1.15	29.14 ± 1.34$^{\Delta}$	32.34 ± 1.19$^{\Delta}$	39.22 ± 2.36$^{**\Delta}$	49.08 ± 3.32$^{**\Delta}$
MCHC (g/dL)	45.32 ± 2.81	43.040± 3.61$^{\Delta}$	33.62 ± 1.59$^{**\Delta}$	29.70 ± 2.149$^{**\Delta}$	25.76 ± 1.89$^{**\Delta}$
Platelets (103/ mm^3)	32.230 ± 0.21	37.28 ± 1.08$^{**\Delta}$	45.320 ± 2.13$^{\Delta}$	52.36 ± 2.25$^{**\Delta}$	61.25 ± 0.055$^{\Delta}$

Unit of measurements: WBCs, RBCs, HCT, MCV, Hb, MCH, MCHC and Platelets values are shown as Mean ± SD (n=5 fish per treatment). Mean with Δ expresses significant intergroup difference while ** show a significant difference (P < 0.05)

Table 2: Biochemical parameters of both control and experimental groups of fish exposed to a sub-lethal concentration of endosulfan

Hematological Parameters	The concentration of endosulfan in ppb				
	0 ppb	1 ppb	3 ppb	5 ppb	7 ppb
Glucose(mg/dl)	141.20 ± 1.924	150.60 ± 2.047$^{\Delta}$	161.20 ± 2.86$^{**\Delta}$	169.60 ± 2.31$^{**\Delta}$	178.60 ± 2.71$^{**\Delta}$
Urea(mg/dl)	15.20 ± 1.30384	13.400 ± 1.14$^{**\Delta}$	10.800 ± 1.303$^{**\Delta}$	08.81 ± 0.60$^{**\Delta}$	6.200 ± 1.31$^{\Delta}$
Total Protein(g/dl)	4.40 ± 0.27	3.900 ± 0.15$^{\Delta}$	3.360 ± 0.54$^{\Delta}$	2.8 ± 0.15$^{\Delta}$	2.20 ± 0.15$^{\Delta}$
Albumin(g/dl)	1.800 ± 0.15	1.520 ± 0.19	1.360 ± 0.11	0.90 ± 0.23	0.600 ±0.15
Globulins(g/dl)	2.380 ± 0.26	2.260 ± 0.21	1.600 ± 0.25	1.16 ± 0.11	0.920 ± 0.19
Cholesterol(mg/dl)	333.80 ± 2.72	245.0 ± 2.73$^{**\Delta}$	257.0 ± 4.47$^{**\Delta}$	263.2 ± 1.92$^{**\Delta}$	279.20 ± 3.96$^{**\Delta}$
Triglycerides(mg/dl)	208.00 ± 10.93	193.20 ± 3.83$^{**\Delta}$	185.60 ± 2.70$^{**\Delta}$	167.55 ± 4.12$^{**\Delta}$	153.40 ± 3.64$^{**\Delta}$
Bilirubin(mg/dl)	0.15 ± 0.01	0.05 ± 0.02$^{\Delta}$	0.11 ± 0.02$^{\Delta}$	0.13 ± 0.01$^{\Delta}$	0.48 ± 0.48$^{**\Delta}$
Enzyme Amylase(µ/l)	126.00 ± 1.58	143 ± 3.34$^{**\Delta}$	174.20 ± 1.31$^{**\Delta}$	193.34 ± 2.95$^{**\Delta}$	213.00 ± 2.55$^{\Delta}$
Calcium(mg/dl)	13.44 ± 0.36	13.36 ± 0.21$^{*\Delta}$	12.40 ± .16$^{**\Delta}$	12.13 ± 0.15$^{**\Delta}$	11.400 ± .31$^{*\Delta}$
Phosphorous(mg/dl)	15.58 ± 0.24	12.48 ± 0.13$^{**\Delta}$	11.18 ± 0.66$^{**\Delta}$	9.44 ± 0.21$^{**\Delta}$	6.34 ± 0.21$^{**\Delta}$

hemoglobin concentration (MCHC) values decreased significantly ($P < 0.05$) in treated group as compared to control group. White blood cells are involved in the regulation of immunological function, the number of (WBCs) increased as a protective response to stress (Pimpao *et al*., 2007). Reduction in the hematological parameters noticed in the present study can be affiliated with osmoregulatory dysfunction, hem synthesis, inhibition of erythropoiesis, rise in the rate of hemolysis or increase lipid peroxidation cause oxidative injury to hemoglobin (Jenkins *et al*., 2003), iron absorption in intestine become reduced (Banik *et al*., 1996). Exposure to endosulfan can cause a reduction in the size of RBCs in fish. Reduction in the size of red blood cells also reduces the amount of space they occupy, which results in a less hematocrit. HCT has used as an essential tool to check-out the effect of pollutants on the health of fish and to check out blood oxygen carrying ability (Singh and Srivastava, 2010). Our studies also show the reduction in the number of red blood cells (RBCs) in *C. carpio* when exposed to endosulfan. According to (Jenkins *et al*., 2003; Seth and Saxena, 2003) when fish is exposed to pesticide it leads to reduction in the hematological value, which indicated anemia, may be due to the haemo synthesis, osmoregulatory dysfunction or due to an increase in the rate of erythrocyte destruction in hematopoietic organs and erythropoiesis. The exposure of endosulfan to Common carp resulted in a significant reduction in hemoglobin values. According to (Sarma *et al*., 2012) a significant decrease occurred in hemoglobin level in freshwater fish due to acute endosulfan exposure (8.1 μg/L) within 12–24 h, after that the values increased & brought back to normal levels at the end of 72 h. However, reduction in hematological parameters, such as HCT (%), hematocrit value, erythrocyte counts, when common carp exposed to endosulfan, and the effects were dependent on the dose (Jenkins *et al*., 2003). The present study showed a significant increase in several platelets as concentration increased. An increase has been observed in the number of platelets when *Ctenopharyngodon idella* is exposed to endosulfan for 96 h. Our experiment showed a decrease in neutrophils, monocytes and lymphocytes values. Similar results were reported by (Modesto and Martinez, 2010) when juveniles of *P. lineatus* exposed to Roundup Transorb (herbicide) for 1 mg L^{-1} and 5 mg L^{-1} for 6, 24 and 96 h, they observed reduction in monocytes number and higher in the number of lymphocytes and leukocytes. These alterations are responsible for the stimulation of the immune system against pollutants, which can be an adaptive response in the organism causing a more influential immune response (Barreto-Medeiros *et al*., 2005).

In our experiment, we observed an increase in the level of blood glucose as noticed by (Das and Mukherjee, 2003) when *Labeo rohita* exposed to cypermethrin for 0.139 ppm for 96 h it led to an increase in the level of glucose.

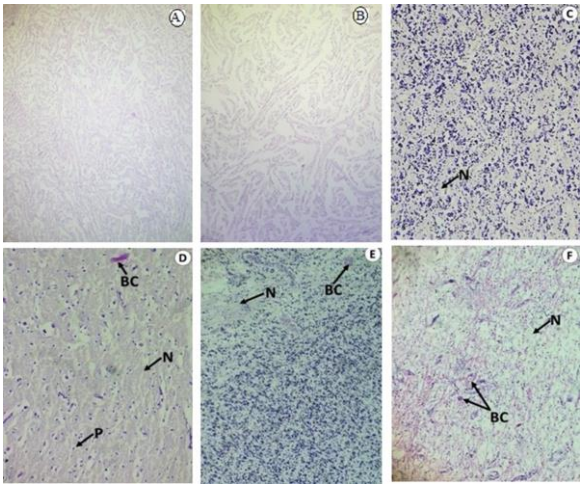

Fig. 3: Histopathological alteration in the brain of *C. carpio*. Necrosis (N), Blood congestion (BC). (**a**, **b**) shows the brain structure of control fish, (**c**, **d**) displays the treated groups I and II, and (**e**, **f**) shows the treated groups III and IV

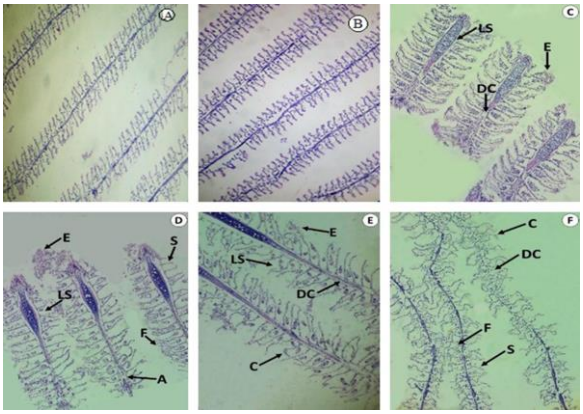

Fig. 1: Histopathological alteration in Gills of *C. carpio*. Shortening of secondary gills lamellae (SL), Epithelial lifting (E), Disruption of cartilage core (DC), Curling (C), Atrophy(A). The fusion of secondary gills lamellae (F). (**a**, **b**) Shows the structure of the gills of control fish, (**c**, **d**) Shows the treated group I, (**e**, **f**) Shows the treated groups II, III and IV

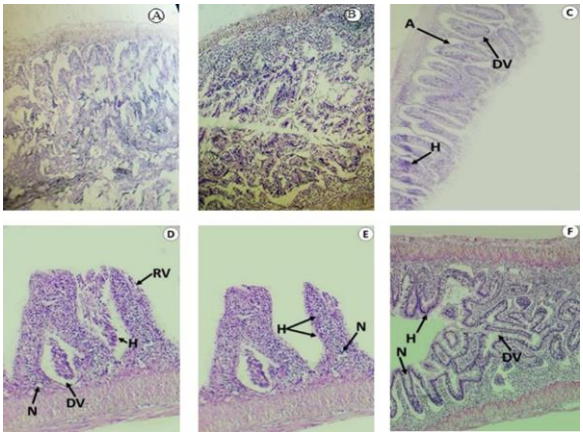

Fig. 4: Histopathological alteration in the intestine of *C. carpio*. Atrophy(A), Detachment of villi (DV), Hemorrhages (H), Detachment of villi (DV) and Necrosis (N). (**a** and **b**) shows the intestine structure of control fish. (**c**, **d**, **e** and **f**) shows Hemorrhages, Necrosis, and Detachment of the villi in intestine tissues of treated fish

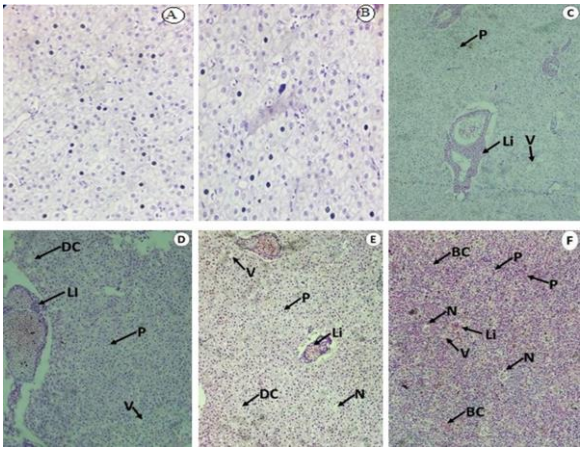

Fig. 2: Histopathological alteration in Liver of *C. carpio*. Pyknosis (P), Vacuolations (V), Dissolution of the cell membrane (DC), Lymphocyte Infiltration (Li), Necrosis (N) and Blood Congestion (BC). (**a**, **b**) Shows a control group of liver structure, (**c**, **d**) Shows the treated groups I and II and (**e**, **f**) Shows the treated groups III and IV

The present study shows a decrease in total protein value in common carp. According to (Jenkins *et al*., 2003) many organisms when they are under stressed conditions, they will mobilize proteins as an energy source using the oxidation of amino acids. Reduction in the protein was featured to stress-mediated immobilization of these compounds to fulfill an increased element for energy by the fish to endure with a condition outside the body. The decrease in protein count was observed in *Clarias gariepinus* when exposed to endosulfan (Yekeen and Fawole, 2011). In this study, there is a significant decrease in globulin and serum albumin level was recorded in treated groups compared to the control group as reported by Jee *et al*. (2005). In our experiment, the Level of urea and triglyceride decreased significantly. Similar results were observed in Triglycerides level (mg/dL) in the blood of the fish *Tilapia mossambica* exposed to sublethal concentration of Cypermethrin (Jipsa *et al*., 2014).

The level of Ca and P was decreased while amylase activity was increased in all the fishes indicating a disturbance in the normal digestive process and may be due to breaking down of the polysaccharides to pure sugar to meet the energy requirement during the pesticidal stress. To define the fish health status, histopathology used as an

economically useful and essential tool and therefore to reflect the health of the whole aquatic ecosystem in the biomonitoring method (Nikalje *et al*., 2012). The given study reflects the sub-lethal effects of endosulfan on histological alterations found in the brain, gills, intestine, and liver of *C. carpio*. Fish gills have a direct connection with the external environment; that is why they are the primary target of contaminants. Gills also perform various relevant and useful functions like acid-base balance, respiration, osmoregulation, and excretion; therefore, in eco-toxicological research gills, morphology can be used as a biomarker (Camargo and Martinez, 2007). In the present research, several histopathological alterations were recorded in gills when fish is exposed to different concentrations of endosulfan. The most visible alterations were a fusion of secondary gills lamellae, disruption of the cartilaginous core, curling also occurred, atrophy and shortening of secondary gills lamellae. Same results were reported by (Roy and Munshi, 1991) in gills structure, separation of epithelium from lamellae, edema, swelling of the epithelial cells and lamellar fusion respectively. The toxic substances can harm the gills of fish, thus leads to breaking the osmoregulatory function of aquatic fauna and also reducing the oxygen expenditure. Several other experiments have acknowledged almost same effects of pesticides on gills of intoxicated fish (Silva and Samayawardhena, 2002). These changes in tissues of intoxicated fish have shown that fish gills are a valid indicator of water quality. According to Alazemi *et al*. (1996) the external location of gills and large surface area, water pollutants can quickly attack fish gills.

The liver is an essential tissue of the fish body, and the liver plays a vital role in the metabolism of lipids, carbohydrates, and protein. The liver is also the storage site of some nutrients and performs the function of detoxification. The function of the liver is to reduce toxic compounds, but its controlling mechanisms can be affected by a high amount of these compounds and could finally lead to structural harm (Brusle and Anadon, 1996). Different structural abnormalities were reported in current research such as Pyknosis, Blood congestion, Vacuolations, Lymphocyte infiltration, and Necrosis. Similar results have been found in *Rhamdia quelen* when exposed to glyphosate (Murussi *et al*., 2016). Our observation and the results of (Mossa *et al*., 2015), report the same alterations in the liver of male albino rats when exposed to fipronil. The alterations in liver histopathological showed necrosis of some hepatic cells, fatty degeneration and focal gathering of lymphocytes.

In the brain, various regions control many functions related to breathing, vision, salt intake, feeding and signals from the lateral line and reacting to the other organs in the body. Endosulfan exposure induces changes in the brain of *C. carpio*. Various alterations were observed in the brain of intoxicated fish such as clogging of blood, necrosis, pyknosis, in brain tissues. Our study is in agreement with (Loganathan *et al*., 2006), noticed the histological alteration in the brain due to zinc toxicity to *L. rohita* exposed 10 mg L^{-1} cause necrosis of neuronal cells of the cerebrum. Same histopathological changes were also observed by (Badgujar *et al*., 2015). The intestine is a sensitive organ that devours any pollutants in the body because it is the main organ that comes into contact with food contaminated with poison. In this study, histopathological changes also occurred in *C. carpio*, such as necrosis, nuclear pyknosis, villus rupture, and excessive goblet cell formation. We also observed hemorrhages, a detachment of villi and necrosis at various points of intestinal tissues. The control group, an intestinal section of *C. carpio*, offered healthy structure with no alteration. The standard architecture of villi consists of muscularis mucosa, lamina properia, stratum compactum, serous membrane, and mucosal epithelium.

In treated groups, I exposed to 1 ppb of endosulfan showed villous shedding, atrophy and hemorrhages, group II exposed to 3 ppb of endosulfan, it caused vitilour shedding, hemorrhage, villous shedding and necrosis, and the groups III and IV exposed to 5 and 7 ppb of endosulfan noticed different histopathological changes detachment of villi, necrosis, and hemorrhages. The predominant changes in intestinal tissue of *C. carpio* with endosulfan toxicity were necrosis, hemorrhages and villi detachment. Our results are supported by (Glover *et al*., 2007), they noticed disruption, the formation of vacuoles in villi of the intestine, fusion of villi during acute toxicity of endosulfan in Atlantic salmon (*Salmo salar*). Our results are further strengthened by (Cengiz *et al*., 2001) when *Gambusia affinis* exposed to different concentrations of endosulfan. Similar results were reported in *Rhamdia quelen* when exposed to glyphosate by (Murussi *et al*., 2016). The results reported in previous on *Rasbora daniconius* and *C. punctatus* support our finding (Cengiz and Unlu, 2006). The endosulfan concentration significantly increases the intestinal necrosis, villi detachment, and hemorrhages in common carp.

Conclusion

From the current study, it is concluded that endosulfan is highly toxic to Common carp causing hematological, biochemical and histopathological changes in various fish organs such as brain, liver, intestine, and gills.

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[Received 05 Jan 2019; Accepted 10 May 2019; Published (online) 20 Aug 2019]