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Studies on toxicological responses of the herbicide paraquat dichloride on *Monopterus cuchia* (Hamilton)

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Abstract

Paraquat dichloride is considered to be highly toxic in nature and is widely used in India. The present work aimed to investigate the toxicity induced by Paraquat on *Monopterus cuchia* exposed to the sub-lethal concentrations of 2 mg/L and 4 mg/L respectively. The study was conducted with emphasis on the haematological parameters, antioxidant enzyme assays and histopathology. The total erythrocyte count, hemoglobin concentration and MCH showed significant reduction, while the total leucocyte count significantly increased by 1.5 fold and 1.83 fold in low and high dose respectively compared to control. The study showed reduction in the activities of superoxide dismutase in liver, kidney and intestine in both the treated groups. However, there was a significant reduction in the activity by 2.98 fold in liver, 2.45 fold in kidney and 1.73 fold in intestine respectively in the high dose compared to control. Significant reduction in the activities of catalase and reduced glutathione in comparison to the control was observed. The catalase activity was reduced by 2.37 fold in the liver, 2.67 fold in the kidney and 3.54 fold in the intestine in the group treated with high dose. However, glutathione-S-transferase activity was found to increase in liver, kidney and intestine with significant increase by 2.28 fold in kidney as compared to control when treated with high dose. Histopathology of liver, kidney and intestine exhibited alterations in structures in treated groups. Thus, the changes observed during the study period establish that Paraquat is highly toxic and thereby its use should be restricted.

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Introduction

Paraquat dichloride, which is chemically known as N, N′-dimethyl-4,4′-bipyridinium is a broad-spectrum contact herbicide and a powerful desiccant (Carlos *et al.*, 1995). Since its manufacture in the year 1962, Paraquat continues to be the third most widely used herbicides in the world and indisputably one of the most frequently employed weed killer in India, especially in the states of Andhra Pradesh, Arunachal Pradesh, Assam, Madhya Pradesh, Telangana and West Bengal (Kumar, 2015). The primary reason for its much acclaimed popularity may be conferred to its broad usage in terms of staple crops like wheat, soya, rice, corn and potatoes (Gwathway and Craig, 2016) even showing its applicability in the instances of fruits such as oranges, apples and bananas, as well as export crops like coffee, tea, cocoa, cotton, sugarcane and rubber. It is classified as highly toxic on the basis of its inhalation toxicity (EPA toxicity class I), as moderately toxic via the oral route (class II), and as slightly toxic by means of the dermal route (category III) (Schenker *et al.*, 2004).Chemically, it belongs to the group of bipyridilium herbicides and is an organochlorine herbicide (Siakpere *et al.*, 2007). It destroys plant tissues by rupturing cell membranes, predominantly due to the production of superoxide radical ion (O_2^-) (Bus *et al.*, 1974).

It has also caused immense health alterations in human beings as well. Pulmonary fibrosis is a striking reality and a leading cause of death in Paraquat poisoning (Ma *et al.*, 2014).Research has proven that its toxicity is mediated by the production of certain free radicals, which cause oxidative damage to the cells (Figueiredo-Fernandes *et al.*, 2006). An additional fact relating to the far-reaching use of Paraquat in agricultural practice throughout the world is that it can very easily enter the water bodies as a result of rain and soil leaching when used in the vicinity of aquatic ecosystems and can therefore compromise the delicate biological balance in those ecosystems, since it is highly water soluble in nature (Ladipo*et al.*, 2011).

Regrettably, Paraquat toxicity studies have been primarily executed in mammals, whereas their possible toxicological effects on fish have received relatively little attention. Therefore, analysis and development of biomarkers of exposure are undoubtedly valuable in evaluating the toxicity of Paraquat contaminated water bodies (Parvez and Raisuddin, 2006). It is also interesting to note that Paraquat toxicity studies have almost never been conducted on *Monopterus cuchia*, which is a widely distributed freshwater eel species, native to Asia, and which has been constantly used as a model animal for eco-toxicological studies since it can easily acclimatize to laboratory conditions. Although the air-breathing fishes are economically very important in India, they are being continuously exposed to the ever increasing number of contaminants, which adversely alter their health, growth, maturity and metabolic aspects(Jha, 1999).

Monopterus cuchia it is a type of swamp eel that inhabits the low lying areas at different sites in the state of Assam. Due to such low lying areas being very much in the proximity of agricultural fields, they are heavily exposed to Paraquat toxicity in its natural habitat (Das *et al.*, 2015).

In view of the fact that data on the toxicological effects induced by Paraquat dichloride on *Monopterus cuchia* is insufficient and also because the usage of this herbicide has drastically increased over the years, the present work aimed to investigate whether the hematological and biochemical indices along with the alterations in the histoarchitecture of the organs were compromised on the fresh-water eel *Monopterus cuchia* exposed to sub-lethal doses of the herbicide.

Materials and methods

Animals

Healthy *Monopterus cuchia* weighing 200 $\pm$ 10 g body mass and of the same size ranging from 30-40cmwere acclimatized in the laboratory for approximately 1 month at a temp of 28 $\pm$ 2^{o}C in plastic aquarium, using tap water of pH= 7.4 $\pm$ 0.1,

and exposing it to 12 hour:12 hour day and night photoperiods prior to being used for experimentation. Dried fish powder and rice bran (5% of the body mass and in the ratio of 5:1) were prearranged as food while the water was religiously changed on alternate days. No sex differentiation of fish was carried out while performing the experiments. Food was withdrawn 24 hrs before each experiment.

Experimental design

The commercial formulation of CLEAR manufactured by Plant Remedies Pvt. Ltd. India (Paraquat Dichloride 24% SL) was used to prepare the test solution. The test procedure was arranged according to the standard method proposed by OECD guidelines (OECD, 1992). For the purpose of each experimental period (96 hour LC_{50}), toxicity test was run, wherein one group of fish (N=6) was exposed only to water (control), while the other 5 groups of fishes were exposed to concentrations that ranged from 20mg/l, 40mg/L, 60mg/L, 80mg/L and 100 mg/L of Paraquat dichloride. The mortality of the fish was recorded at logarithmic time intervals, i.e after 6, 12, 24, 48, 72 and 96 h of exposure.

Based on the results of the 96 hour LC_{50} value determination of Paraquat dichloride, six fishes in each group were exposed for 7 days to the nominal concentration of 2 mg/L and 4 mg/L, which were 1/10th and 1/20th value of LC_{50}.

After the exposure period, the fishes were removed from the plastic buckets, immediately anaesthetized by benzocaine (0.1 g/L) and caudal vein blood was drawn with a heparinised syringe. The animals were then killed by excision of the spinal cord behind the operculum and the targeted tissues (liver, kidney, and intestine) were removed and promptly placed on ice and frozen at -70^{o}C for analysis.

Hematological parameters

Total count of RBC**:** The RBC count was carried out using the Improved Neubauer haemocytometer (Shah and Altindag, 2004). The total number of erythrocytes was reported as 10^6/ mm^3 (Wintrobe, 1967).

Total count of WBC: WBC count was carried out using the Improved Neubauer haemocytometer (Shah and Altindag, 2005). The total number of WBC was calculated as mm^3x10^3.

Determination of haemolglobin:Whole blood Haemoglobin was assessed by the Cianmethhaemoglobin method in a spectrophotometer at 540 nm.

The mean corpuscular Haemoglobin (MCH) was calculated using standard formulae as described by Jain, 1993.

MCH = (Hb in g / RBC in millions) × 10 pg

Antioxidant enzyme assays

Superoxide dismutase (SOD): Copper-zinc superoxide dismutase (CuZn-SOD) activity was ascertained by the method of Flohe and Otting, 1984. SOD activity was expressed in U SOD mg of $protein^{-1}$, with one U of SOD equivalent to the quantity of enzyme that promoted the inhibition of 50% of the reduction rate of cytochrome c.

Catalase (CAT): CAT activity was ascertained according to the technique described by Beutler, 1975 by monitoring the H_2O_2 decomposition from the decrease of absorbance at 240 nm. The activity of CAT was expressed in µg/ml/min.

Glutathione-s-transferase (GST): GST activity was ascertained according to the methodology proposed by Keen *et al.,* 1976, following the complex of Reduced Glutathione (GSH) with 1-chloro-2,4-dinitrobenzene (CDNB) at 340 nm, which is expressed asµg/ml/min.

Non-enxymatic antioxidant assay

Reduced glutathione (GSH): GSH level were ascertained according to the methodology by Beutler *et al.,* 1963 using 5,5–dithiobis–2– nitrobenzoic acid (DTNB), following Monterio *et al.,* 2009 and Thomaz *et al.*, 2009. The supernatant of the extract was added

to 0.25 mM DTNB in 0.2 M Potassium Phosphate Buffer (pH = 8.0) and the formation of thiolate anion was determined at 412 nm absorbance against a GSH standard curve. The GSH content was expressed as µg/g/wet wt of tissue.

Statistical analysis

Experiments were conducted in triplicates. The data that was composed from different replicates were statistically analysed and were presented as mean ± S.E.M. Values of RBC, WBC counts, Haemoglobin concentration, MCH value and the different enzymes were compared statistically with control using one way ANOVA with the help of Microsoft EXEL and ORIGIN 6.1. Differences with $P<0.05$ were deemed as statistically significant.

Tissue histopathological analysis:

At the end of the experimental period on the 7th day, three fishes per treatment groups (2 mg/L and 4 mg/L) were sacrificed and dissected. The tissues (liver, kidney and intestine) collected were washed using buffer normal saline and fixed using Carnoy's fixative for 48 hrs. After washing, the tissues were dehydrated using successive percentages of graded alcohol series (30, 50, 70, 90 and 100%). The tissue samples were thereafter cleared with xylene and then embedded in paraffin wax at 58°C to 60°C melting point. Sections were produced using a rotary microtome (RADICAL RMP-30) at 4 micron thickness. The cut sections were then stained with haematoxylin-eosin. All the slides were examined under a light microscope (Leica DM- 6000, Germany) and the photomicrographs were taken at 400 magnification under a microscope at its highest resolution (Banaee *et al.*, 2013a, b).

Results

Determination of LC_{50} value

When the fishes were exposed to Paraquat dichloride, it induced death of the fishes in a concentration dependent manner. 100 mg/L, the highest concentration tested in this study, was found to be the deadliest and it induced death of the fishes within 48 h, while 100% mortality was observed at 96 h. At a concentration of 20 mg/L, out of a probable 10 fishes, 2 of the fishes reported mortality after 96 h. The LC_{50} value of the experimental set up was determined at a concentration of 40 mg/L.

Table 1. Blood parameters of *Monopterus cuchia* exposed only to water (control), to 2 mg/L of Paraquat Dichloride (24% SL) and 4 mg/L of Paraquat Dichloride (24% SL) for 7 days.

Parameter	Exposure time	Control	Concentration of paraquat dichloride in	
			2 mg/L	4 mg/L
RBCs(x10^6/mm^3)	7 days	6.5±0.145	3.71±0.078*	1.27±0.028*
WBCs(x10^4/mm^3)	7 days	5.68±0.116	8.48±0.158*	10.4±0.104*
Hemoglobin(g/dL)	7 days	14±1.5	9.2±1.2*	7.0±1.0*
MCH(pg)	7 days	21.53±1.89	14±1.75*	10.44±1.6*

However, during the test with sub lethal concentrations (2 mg/L and 4 mg/L), there was no fish mortality in any of the experimental groups and the water parameters were monitored and kept constant. In all cases, control groups of fishes were maintained (Fig. 2.).

Haematological parameter

The results of the hematological parameters i.e. the mean RBC, WBC, hemoglobin and derived erythrocyte indices (MCH) of *Monopterus cuchia* are shown in Table. 1. A pronounced decrease in hemoglobin concentration and the number of erythrocytes in the fish group treated with 2 mg/L and 4 mg/L were found after 7 days of exposure as compared to control. A significant increase in the

total number of leucocytes was observed. MCH value was also significantly decreased in the treated batch.

Enzyme activity

The primary antioxidant enzymes i.e. SOD and CAT activities showed a significant decrease in the liver, kidney and intestine of the treated fish in both low and high concentrations (i.e. 2 mg/L and 4 mg/L) as compared to the control (Fig. 3. and 4.). In contrast, GST activity was significantly increased in the treated fishes (Fig. 3.).

Alternatively, a decrease in the level of the non-enzymatic antioxidant GSH was found in both the treated groups as compared to control (Fig. 6.).

Histology

Liver morphology of Monopterus cuchia (control)

The present study demonstrates that the liver of control *M. cuchia* exhibited a normal histo-architecture and there were no major pathological abnormalities noticed.

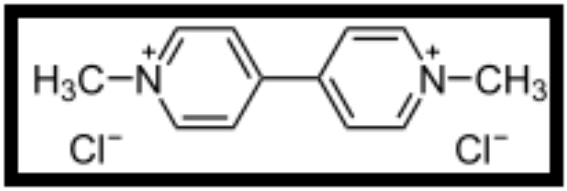

Fig.1. Chemical structure of paraquat dichloride.

The volume of the liver in case of *M. cuchia* is fairly large, which is proportionally acceptable when compared to its body volume. Cord-like structures are observed in the liver of the control batch, which are formed by the roundish polygonal hepatocytes that are limited at one side by the liver capillaries or narrow sinusoids. Central to each cord are thin bile canaliculi adjacent to the hepatocytes.

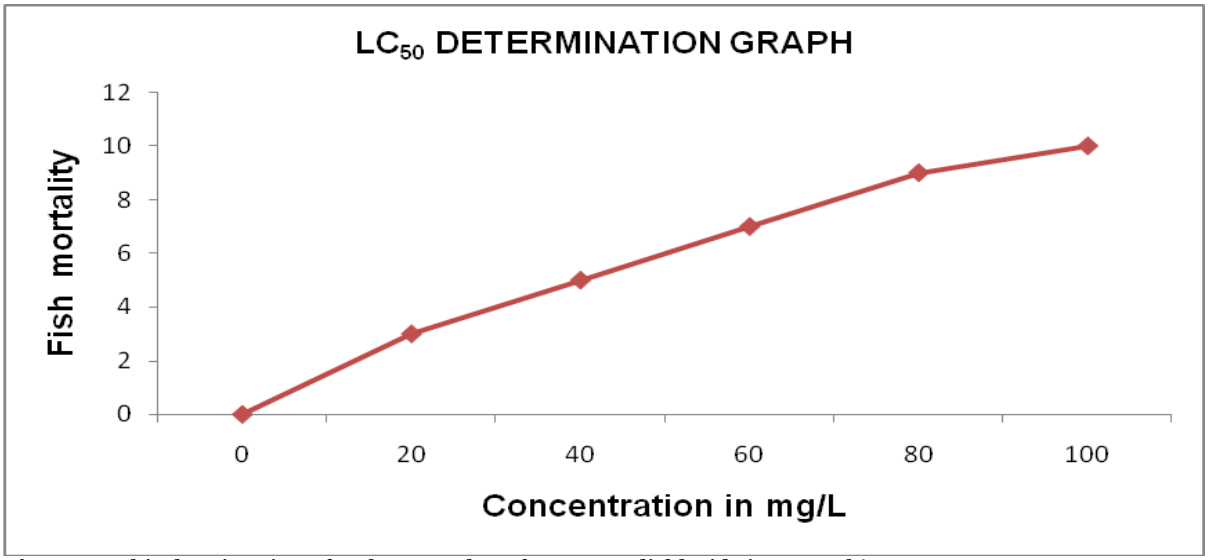

Fig. 2. Graphical Estimation of 96 h LC_{50} value of Paraquat dichloride in *M. cuchia*.

The boundaries between the hepatocytes are easily discernible. The nucleus is proportionally big, spherical and centrally placed while the cells showed a homogenous cytoplasm (Fig. 7.).

Liver morphology of Monopterus cuchia exposed to treated concentrations (2 mg/L and 4 mg/L) of paraquat dichloride

The liver of *Monopterus cuchia* in treated condition presented some indisputable pathological changes when compared to the control. Superficially itself, the size of the liver relatively increased in the fishes exposed to Paraquat dichloride.

The liver cells were observed to be swollen; the cell outline becoming indiscernible. Some nuclei shifted to the lateral position in the cell, showing variable shape and size.

Another change noticed when the fish liver tissue was exposed to the low concentration (2 mg/L) of Paraquat dichloride was the increase in the diameter of the blood sinusoids. Cytoplasmic vacuolization's were observed at many places, clearly indicating the

initiation of mild necrosis of the organ. Melano-macrophage aggregation was also observed (Fig. 8A., 8B.).

The herbicide brought about extended regions in the liver to be totally necrotic and hemorrhagic. Hepatocytes varied in their morphology, some showing lateral nuclei, some clearly signifying degeneration, while others were highly pycnotic. Almost certainly due to cytolysis, permeation of macrophages or phagocytes increased in these regions.

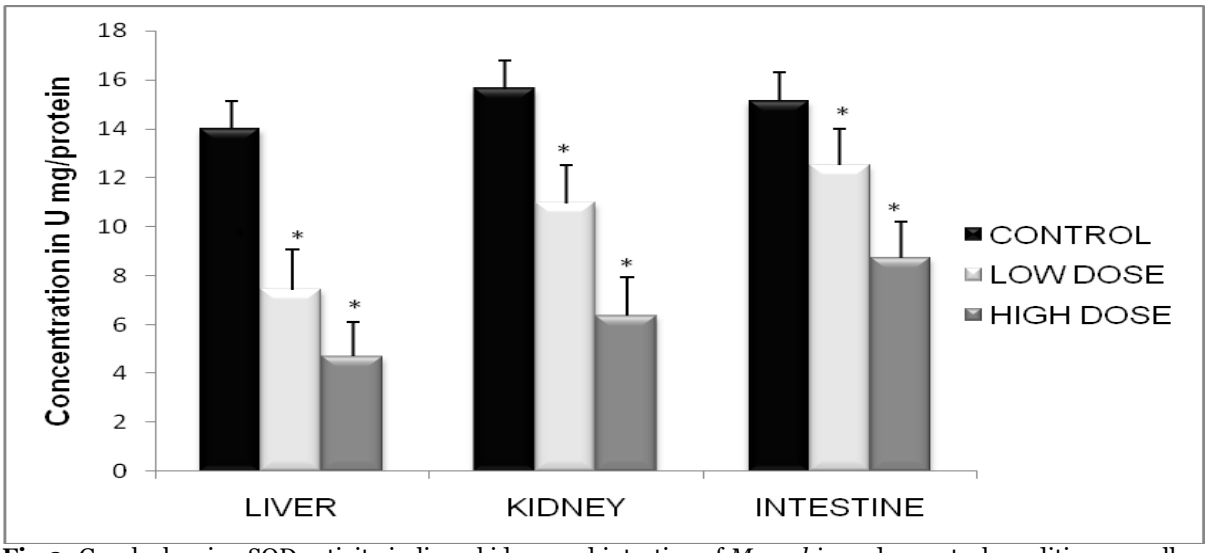

Fig.3. Graph showing SOD activity in liver, kidney and intestine of *M. cuchia* under control condition as well as under low (2 mg/L) and high concentration (4 mg/L) of Paraquat dichloride exposure. Values are significant at $P < 0.05$. (* indicates that the values are significantly different at $P < 0.05$ level compared to the respective control values determined by one way ANOVA analysis).

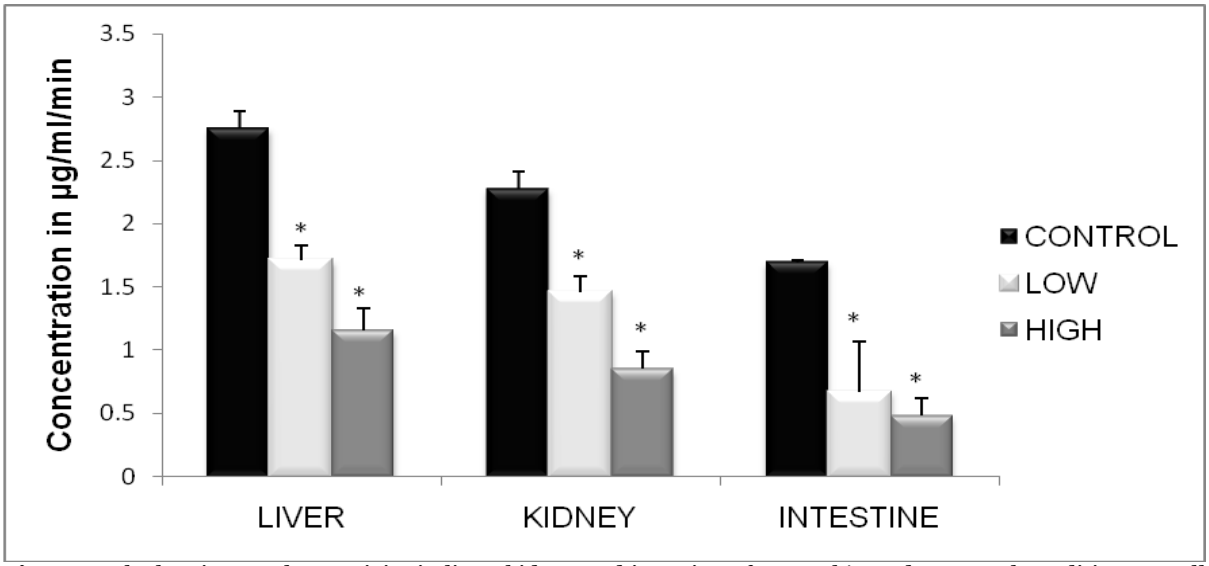

Fig.4. Graph showing catalase activity in liver, kidney and intestine of *M. cuchia* under control condition as well as under low (2 mg/L) and high concentration (4 mg/L) of Paraquat dichloride exposure. Values are significant at $P < 0.05$. (* indicates that the values are significantly different at $P < 0.05$ level compared to the respective control values determined by one way ANOVA analysis).

The damage under the exposure to a high dose of Paraquat dichloride comprised of hypertrophy of hepatocytes, nuclear hypertrophy, higher cytoplasm vacuolization, blood congestion in the central veins,

as well as the diffusion of melano-macrophages in the parenchymal tissues of liver (Fig. 9A., 9B.).

Morphology of kidney of Monopterus cuchia (control)

The kidneys of a teleost species are one of the foremost organs to be affected by contaminants in the water. Teleost kidney structure is reflected by its complicated arrangement involving many different cell types and an intricate vascular system. Kidney of control fish showed normal histoarchitecture composed of numerous corpuscles with well-defined glomeruli, Bowman's capsule and a system of renal tubules (Fig. 10.).

Kidney morphology of Monopterus cuchia exposed to treated concentrations (2 mg/L and 4 mg/L) of paraquat dichloride

Various changes were observed in the Paraquat treated kidney of *M. cuchia*. At a concentration of 2 mg/L of the herbicide, alterations such as distortion of Bowman's capsule, glomerular cell distortion, fusion of renal tubules, as well as tubular cell distortion was reported (Fig. 11A.).

However, at a concentration of 4 mg/L i.e. at exposure to high dose of Paraquat dichloride more severe changes were noticed.

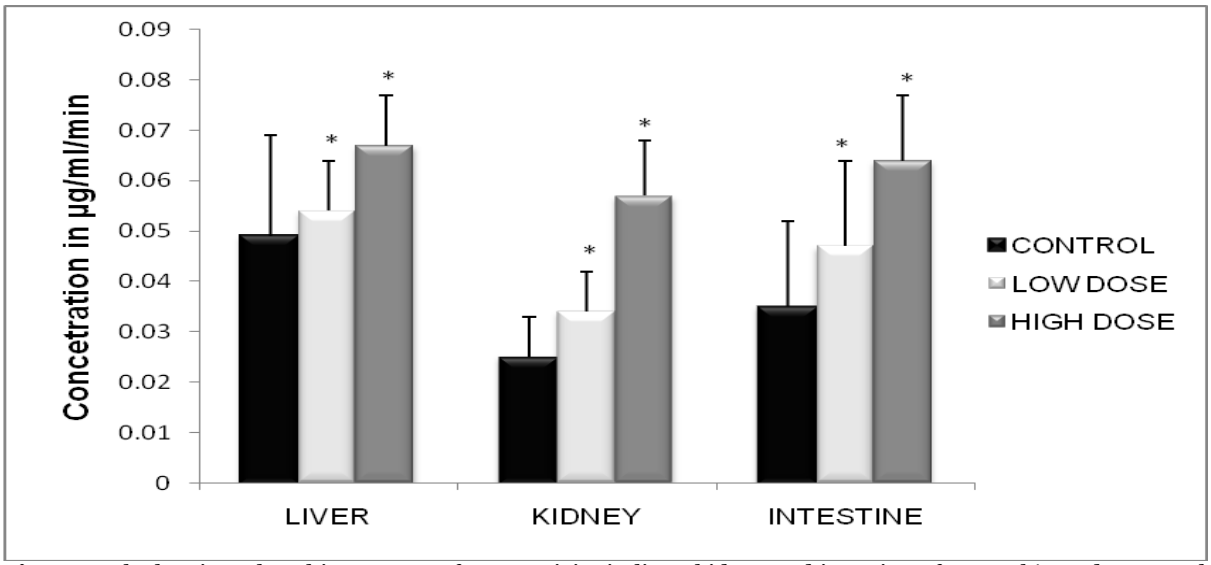

Fig.5. Graph showing Glutathione-S-transferase activity in liver, kidney and intestine of *M. cuchia* under control condition as well as under low (2 mg/L) and high concentration (4 mg/L) of Paraquat dichloride exposure. Values are significant at $P < 0.05$. (* indicates that the values are significantly different at $P<0.05$ level compared to the respective control values determined by one way ANOVA analysis).

These changes include melano-macrophage aggregate, picnocytic nucleus, enlargement of renal tubule, tubular cell disarrangement, as well as the rupture of renal tubules (Fig. 11B.).

Morphology of intestine of Monopterus cuchia (control)

The intestine plays an important role in osmoregulation and in the maintenance of the body's ion and water balance.

The intestine of the control *Monopterus cuchia* presented normal architecture, showing well organized villi, columnar absorptive cells, goblet cells and the mucosal layer (Fig.12.).

Morphology of intestine of Monopterus cuchia exposed to treated concentrations (2 mg/L and 4 mg/L) of paraquat dichloride

As is evident from the comparative revelations between the control and the treated photographs of the intestine of *Monopterus cuchia*, severe atrophic degeneration can be observed. The mucosa lost its usual shape and normal architectural plan. Villi revealed altered orientation and were disorganized, greatly reduced, flattened and inflamed at the base. Their tips were found ruptured at places leading to the exudation of mucus in lumen.

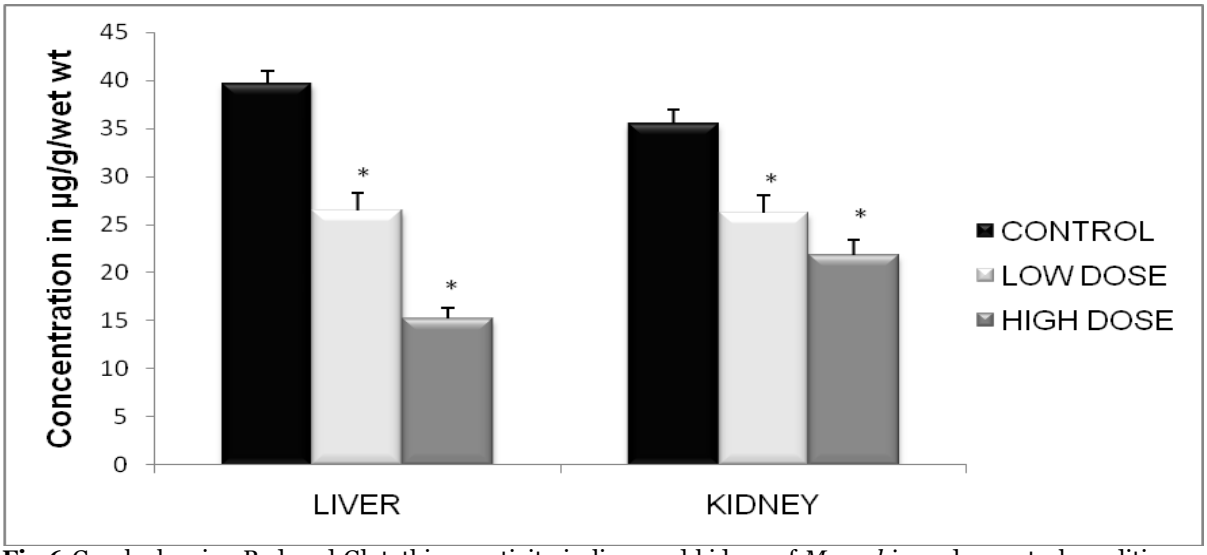

Fig.6. Graph showing Reduced Glutathione activity in liver and kidney of *M. cuchia* under control condition as well as under low (2 mg/L) and high concentration (4 mg/L) of Paraquat dichloride exposure. Values are significant at P<0.05. (* indicates that values are significantly different at P<0.05 level compared to the respective control values determined by one way ANOVA analysis).

The nuclei showed disintegration and occupied the apical position contrary to the basal one in control. Autolysis of cells was observed, leaving clear spaces suggesting oedema.

The current study also reported vacuolation of columnar epithelial cells. Swelling of cells as well as blood hemorrhage was observed in the current study, all of which are indicative of disruption caused by exposure to the herbicide Paraquat dichloride (Fig. 13, 14A. and 14B.).

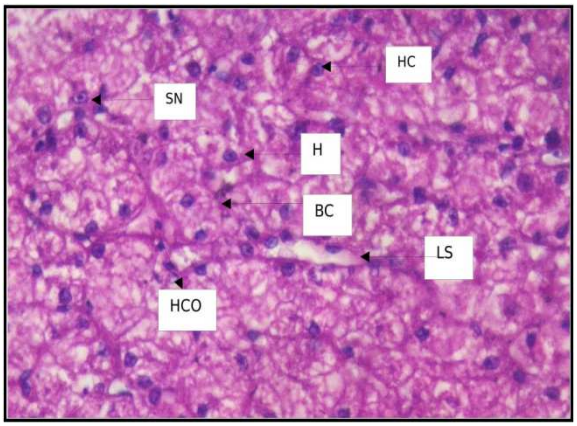

Fig.7. Histoarchitectural changes observed in the T.S. of liver (H and E stain, x400) of *M. cuchia* (control) showing polygonal hepatocytes (H), spherical nucleus (SN), bile canaliculi (BC), hepatic chords (HCO), homogenous cytoplasm (HC) and liver sinusoids (LS).

Discussion

Haematological parameter

Alterations in the hematological parameters can be the outcome of the activation of the fish immune system in response to the herbicide, which in turn may be an adaptive response of the organism resulting in a more effective immune defense(Barreto-Medeiros *et al.*, 2005).

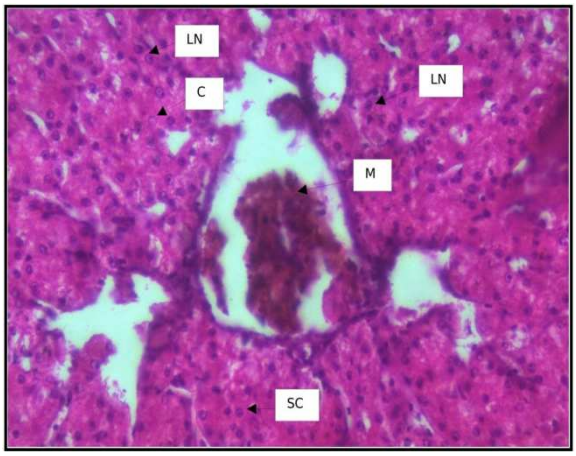

Fig.8A. Histoarchitectural changes observed in the T.S. of liver (H and E stain, x400) of *M. cuchia* exposed to low concentration (2 mg/L) of Paraquat dichloride showing lateral nuclei (LN), cellular vacuolization (CV), melanomacrophage aggregate (M), Swollen hepatocytes (SC).

Since the red blood corpuscles are the oxygen carrying conduits of the body, the quantitative decrease in their levels might have lead to a decrease in the respiratory potential of the tissues.

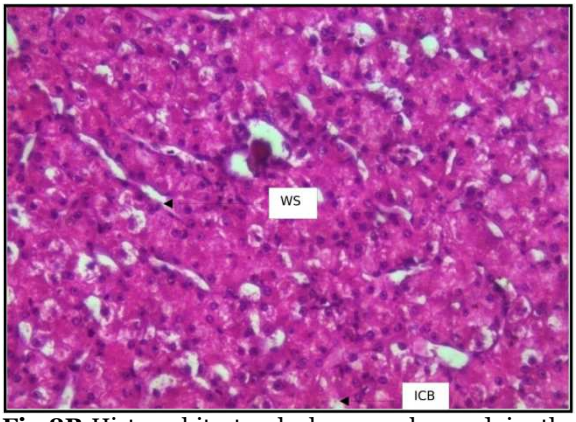

Fig.8B. Histoarchitectural changes observed in the T.S. of liver (H and E stain, x400) of *M. cuchia* exposed to low concentration (2 mg/L) of Paraquat dichloride showing indistinguishable cell boundary (ICB) and widening of sinusoids (WS).

These findings corroborate with the diminished body movements and signs of lethargy observed in the treated group of fish. Similar results like a significant decrease in RBC count leading to anaemia as a result of inhibition of erythropoiesis and increase in the rate of erythrocyte destruction in haemopeotic organs have been made by Goel *et al.*,1980.

Natarajan, 1981 reported a reduction in Hb content, RBC count and PCV values, resulting in hypochronic anaemia due to deficiency of iron and decreased utilization for Hb synthesis. Such a decrease in RBC and anaemic response has been recounted by Koundinya and Ramamurthy, 1979 in *Sarotherodan mossambicus* and *Tilapia mossambicus* after exposure to lethal concentration of sumithion, and Lai *et al.,* 1986 in *H. fossilis* exposed to malathion. Reduction in the values of these parameters were also reported in *Prochilodus lineatus* exposed to Clomazone (Pereira *et al.*, 2013) and in *Labeorohita* exposed to Fenvalerate (Prusty *et al.*, 2011).

The reduction of HB might be due to the coagulation of blood in the treated fish. Such a decrease in Hb concentration was also observed in *C. carpio* on exposure to pesticide (Nithyanandam *et al.*, 2007). Kathowaska *et al.*, 1985 also reported a similar decrease in Hb concentration and RBC count in Japanese quail induced due to an organophosphorus insecticide. Decrease in MCH reported in this study clearly indicate hypochronicmicrolytic anemia. The above findings are similar to the report made by Shakoori *et al.*, 1996.

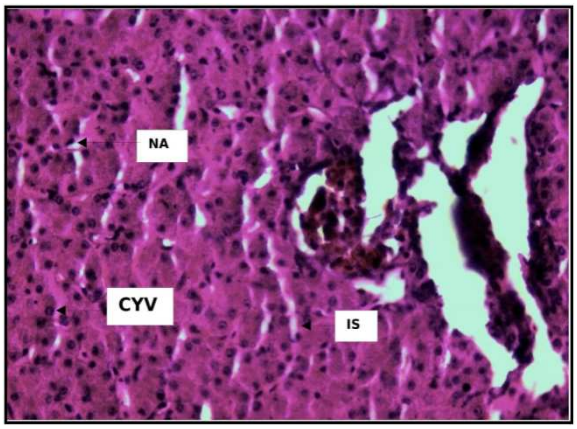

Fig.9A. Histoarchitectural changes observed in the T.S. of liver (H and E stain, x400) of *M. cuchia* exposed to high concentration (4 mg/L) of Paraquat dichloride showing nuclear atrophy (NA), cytoplasmic vacuolization (CYV) and increase of sinusoids (IS).

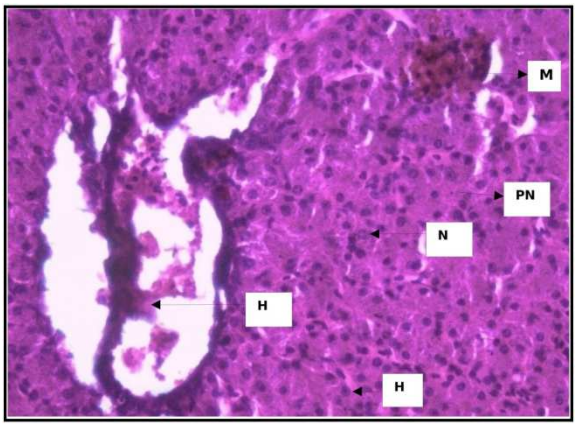

Fig.9B. Histoarchitectural changes observed in the T.S. of liver (H and E stain, x400) *M. cuchia* exposed to high concentration (4 mg/L) of Paraquat dichloride showing melanomacrophage aggregation (M), hepatocyte hypertrophy (HH), hemorrhage from the central vein (H), necrosis of hepatocytes showing multi-nucleated cells (N), and picnocytic nucleus (PN).

The increase in WBC in fish exposed to Paraquat may

be due to tissue damage. The present observation is contrary to the observation made by McLeay, 1973 who suggested that stress is attributable to a decrease in the number of circulating lymphocytes. An increase in total leucocyte count was also accounted by Bhargava *et al.,* 1999 in the fish *Channa striatus* on exposure to BHC and Malathion.

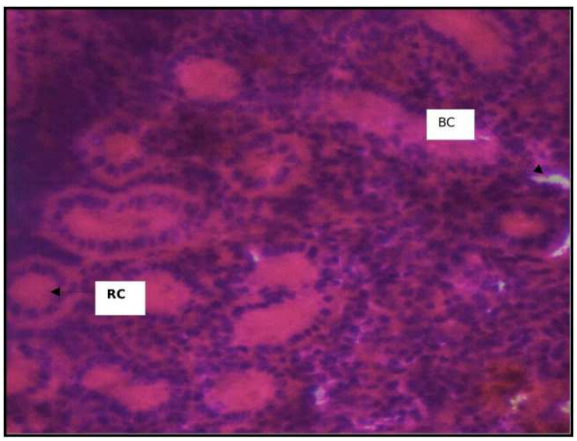

Fig.10. Histoarchitectural changes observed in the T.S. of kidney (H and E stain, x400) of *M. cuchia* (control) showing renal tuules with well orgaized cellular structure (RC) and well-defined Bowman's capsule space (BC).

The results of the experimental trials reveal that Paraquat treated fishes arrive at stage of extreme sluggishness due to severe weakness when exposed to a prolonged period of exposure.

Enzyme activity

The SOD–CAT system is the first line of defense against oxygen toxicity (Pandey *et al.*, 2003), and these enzymes are frequently used as bio-markers, indicating the production of reactive oxygen species (ROS) (Monteiro *et al.,* 2006).

An excess production of hydrogen peroxide may reduce the activity of SOD, while superoxide anion may be responsible for decreased activity of CAT (Vasylkiv *et al.*, 2011). This hypothesis is concurrently established by the fact that the CAT activity was also reduced in the fish after exposure to the herbicide, enhancing the accumulation of hydrogen peroxide in the cell (Modesto and Martinez, 2010). Moreover, this reduction in CAT activity may be due to the formation of superoxide ions, which are almost certainly not being neutralized efficiently by SOD, indicating a failure on its part to provide proper defense against the formation of ROS species.

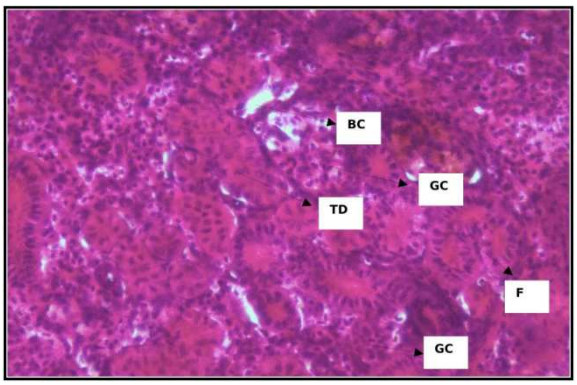

Fig.11A. Histoarchitectural changes observed in T.S. of kidney (H and E stain, x400) of *M. cuchia* exposed to low concentration (2 mg/L) of Paraquat dichloride showing distortion of Bowman's capsule (BCD), Glomerular cell distortion (GCD), fusion of renal tubules (F), and tubular cell distortion (TD).

Thereby, in the present work, inhibition of these two enzymes in the tissues selected for the experimental purpose (liver, kidney and intestine) establishes the interference in the antioxidant defenses of the fish occurring due to the exposure of the fishes to the herbicide.

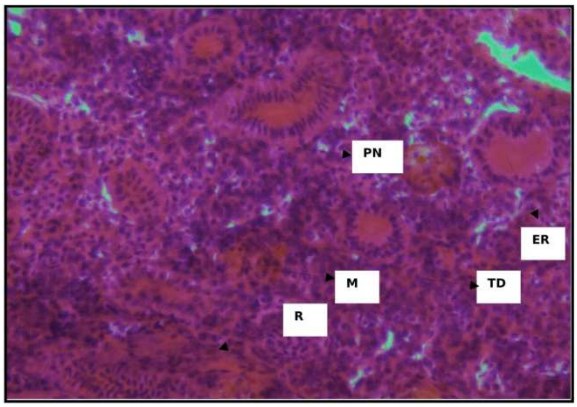

Fig.11B. Histoarchitectural changes observed in T.S. of kidney (H and E stain, x400) of *M. cuchia* exposed to high concentration (4 mg/L) of Paraquat dichloride showing picnotic nucleus (PN), melanomacrophage aggregate (M), tubular cell distortion (TD), enlargement of renal tubule (ERT), and rupture of renal tubule (R).

The glutathione-S-transferases are a family of enzymes which play an imperative role in

detoxification as well as elimination of xenobiotics (Jakoby, 1978). Within the cell, the cytosolic GST can help in the detoxification process either by (a) these enzymes may boost the availability of lipophilic toxicants to microsomal cytochromes P-450 by acting as carrier proteins (Hanson-Painton *et al.*, 1983); (b) glutathione-S-transferases serve the purpose of catalysts for the conjugation of glutathione with electrophiles (Nemoto and Gelboin, 1975). The altered GST in the treated fish in comparison to the control indicates an increase in oxidative stress induced by the herbicide. Adverse conditions may modify the different metabolic events in the tissues, leading to an increase in the endogenous GST level. Similar result was also reported in fresh water fish under mineral contamination (Borvinskaya *et al.*, 2013).

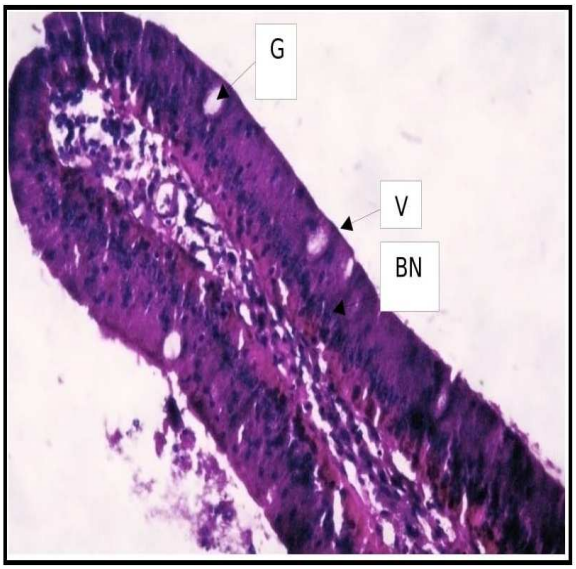

Fig.12. Histoarchitectural changes observed in the T.S. of intestine (H and stain, x400) of *M. cuchia* (control) showing organized villi (V), Goblet cells (G), and position of nucleus of the cells in basal position (BN).

Although intestinal Glutathione-S-transferases are known to be sensitive to dietary pollutants including polycyclic aromatic hydrocarbons(Clifton and Kaplowitz, 1978) results from the present study indicate that intestinal glutathione-S-transferase activity may not be a sensitive indicator for change induced by the herbicide. However, since there was an increase in the GST activity in the liver as well as the kidney in the Paraquat treated fish, it could be concluded that the exposed fishes tried to show a degree of defense against the herbicide.

Non-enzyme activity

Glutathione, which is the major non-protein thiol of cells, operate as a sink for free radicals and other reactive species(Lima, 2004). Variations in cellular glutathione content are considered to be indicators of the degree as well as the duration of exposure to oxidant pollutants in fish (Dautremepuits *et al.*, 2009).

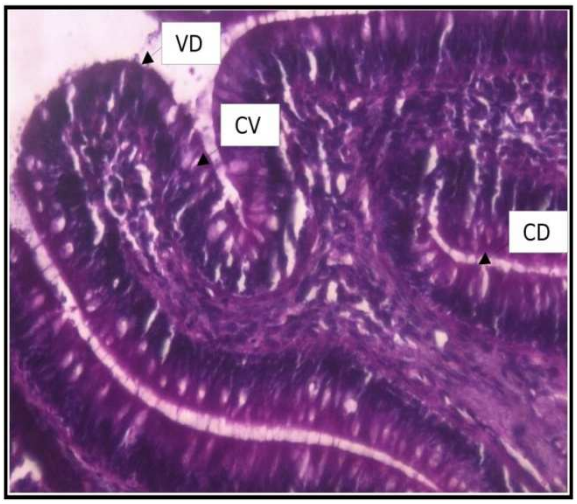

Fig.13. Histoarchitectural changes observed in the T.S. of intestine (H and E stain, x400) of *M. cuchia* exposed to (2 mg/L) concentration of Paraquat dichloride showing disruption of tips of villi (VD), cellular vacuolization (CV) and cellular disruption (CD).

Within cells, GSH is the dominant intracellular thiol and has an important function in the cellular defence against oxidative injury (Meister and Anderson, 1983). Due to the significant lower levels of GSH in the liver of Paraquat treated *M. cuchia* compared to the control, the liver in the treated fish may be less capable in the transformation and excretion of xenobiotics from the body and, consequently, a reduction in detoxifying capacity of the liver may lead to enhanced risk of xenobiotic damage of this organ. Similar reports were also made by Hjeltnes *et al.*,1992 in Atlantic salmon (*Salmosalar).*

However, even though the decrease of GSH in the kidney and intestine are not significantly pronounced, it could nonetheless indicate the failure of the organ

to put forth a strong defense against the chemical. The defensive and adaptive roles of GSH against oxidative stress induced toxicity are well established in aquatic animals (Regoli and Principato, 1995; Otto and Moon, 1995). When not neutralized, reactive oxygen species can interact with membrane lipids (Ahmad *et al.*, 2008), producing lipid peroxidation, which is considered as one of the main consequences of oxidative stress.

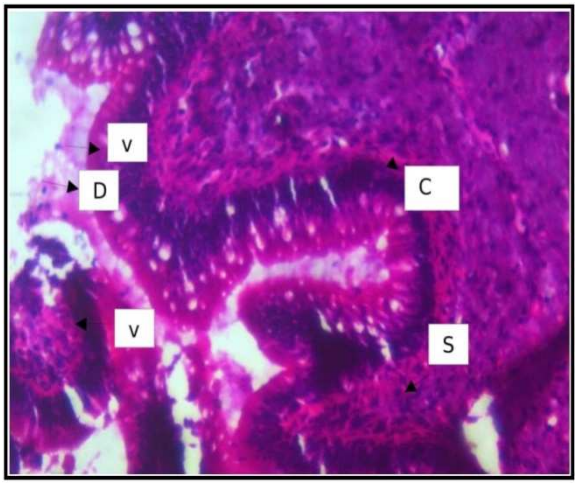

Fig.14A. Histoarchitectural changes observed in T.S. of intestine (H and E stain, x400) of *M. cuchia* exposed to high concentration (4 mg/L) of Paraquat dichloride showing the following changes – V (cellular vacuolization of the columnar absorptive cells), D (disruption of villi organization), CD (cellular disruption), S (swelling of cell).

Histopathology

The utilization of histopathological biomarkers is one of the best procedures for the evaluation of the effects of pollutants on aquatic ecosystems as interruption of living processes at the sub-cellular levels by xenobiotics can lead to cell injury, resulting in degenerative diseases in the target organs(Pacheco and Santos, 2002).

Necrosis coupled with structural destruction of liver cells in these fishes clearly shows the effect of Paraquat in the destruction of the cellular membrane of liver cells. In other words, liver is a place for multiple oxidation reactions and it is in the liver that production of most of the free radicals in body take place. Therefore, there is a probability that due to lipid peroxidation, the cellular membrane gets destroyed, inhibiting intracellular oxidative phosphorylation (Zaragoza *et al.*, 2000).

Due to the disturbance in osmotic regulation of biological membranes, the volume of the nuclei and nucleoli increase and this ultimately leads to necrosis of the liver cells. Extensive pathological changes in the tissues of liver of fish treated with Paraquat can disturb homeostasis and lead to further physiological disorders. Similar results were also reported in *Brachydanio rerio* after exposure to sub lethal levels of the organophosphate Dimetoato 500(Rodrigues and Fanta, 1998).

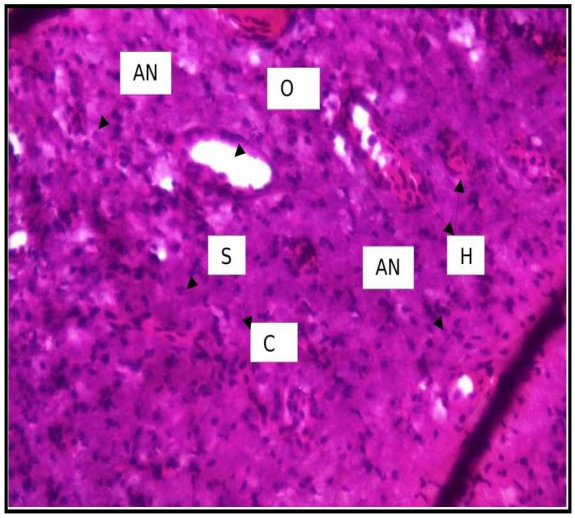

Fig.14B. Histoarchitectural changes observed in T.S. of intestine (H and E stain, x400) of *M.cuchia* exposed to high concentration (4 mg/L) of Paraquat dichloride showing the following changes – AN (Apical nuclei), OD (oedema), S (swelling of cell), H (hemorrhage), CV (cellular vacuolization).

The increase in the size of urinary tubules of the kidney that was observed in this study infers to hydropic swelling. The appearance of this lesion was very much similar to the observation in Nile tilapia (*Oreochromis niloticus)* exposed to contaminated sediments (Peebuaa *et al.*, 2006). This alteration can be corroborated by the occurrence of cellular hypertrophy and the presence of small granules in the cytoplasm. The presence of tubular degeneration, coupled with the initiation of necrosis of the tissue in the present study indicates that the kidney suffered damage after exposure to Paraquat dichloride. Elevated concentration of Paraquat caused shrinkage of glomeruli and blood hemorrhage. This may lead to

cellular degeneration as well as enhancement in the amount of edematous fluid in the interstitial space. The capillaries of Bowman's capsule were also observed to be disorganized. As a result, the nephrons were not so active in its filtration role, showing diminished functional efficiency of the organ.

Prolonged treatment exhibited swelling in the luminal portion of villi along with the appearance of vacuolated epithelial cells. Since the function of villi in the absorptive capacity of an animal is very important, the damage at this site in the present case could lead to the establishment of the fact the extent of disorderliness in the intestinal physiology, including absorption, induced by the herbicide is indeed serious. Other cellular injuries including oedema may be related to the liberation of acid hydrolases from lysosomes leading to cellular autolysis. The rupture in the tips of villi observed in the present study may be considered as a mode of detoxification in response to the herbicide. Related observations have also been made by Moitra and Lai, 1989, as well as by Anitha kumari and Ram Kumar, 1997 in *Channa punctatus* under influence of aquatic pollutants.

The damage of the cells in the intestine negatively affects the appetite of the organism (Khillare, 1985). Hence, the production of energy for various metabolic processes would consequently slow down. Similar observations were also reported in *Channa gachua* exposed to the chemical DDT (Paraveen, 1980). Sometimes, the damage incurred to varied tissues is so severe that failure or varied disorders in their function can even lead to the death of the fish.

The study was thus conducted to integrate a much needed understanding of the traumatizing effects of Paraquat on the targeted model organism, as they represent not only themselves but the other breeds of fauna in the different aquatic ecosystems that have been besieged by Paraquat. Paraquat dichloride is already banned in many countries around the world. Nevertheless, Paraquat is still one of the world's most widely used herbicides, especially in developing countries like India, where its use leads to the poisoning of thousands of workers.

Seiyaboh *et al*., 2013; Suntres, 2002 and Arivu *et al*., 2016 have indicated that Paraquat is toxic and has the potential to impair the physiological behaviour, morphology, hematology and biochemical activities of a plethora of fish species.

Conclusion

The present work showed that use of Paraquat herbicide is toxic to *M. cuchia* and causes hematologic, enzymatic and histopathological changes in different organs like liver, kidney and intestine. Such effects noticeably have harmful consequences on the fish, and in the long run it may have negative impact on the fish population. This information has a great importance in terms of preservation, considering that this herbicide is prevalent at sublethal concentrations in the natural environment. Thereby, the use of Paraquat at riverside and coastal areas should be strongly controlled and carefully monitored to avoid exposure to aquatic environments.

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