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# Toxicological effect of herbicide paraquat dichloride on histological profile (liver and muscles) of freshwater fish *Cirrhinus reba* (Hamilton, 1822)

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***Abstract:*** *The herbicide paraquat dichloride is being used indiscriminately in herbage areas all across India, and it might end up in nearby freshwater bodies via irrigation and rain. Paraquat dichloride posed a serious threat to aquatic wildlife, notably fish. Therefore, this study was conducted to assess the toxic effects of paraquat dichloride on the biochemical and histomorphological alterations in the muscles and liver of the fish Cirrhinus reba (Hamilton, 1822) over 72 hours at a sublethal dosage of 0.0029 ppm. The biochemical composition of the samples differed significantly. Under herbicidal stress, the overall levels of glycogen, protein, lipid, and alkaline phosphatase (ALP) were reduced, but acid phosphates ACP activity were enhanced in muscle and liver tissues. Biochemical values reveal the intensity of the toxic action of the herbicide. Similarly the effect of Paraquat dichloride exposures were observed in histomorphological changes in muscles and liver. The muscles showed degeneration in muscle bundles accompanied with necrosis as well as atrophy and vacuolar deterioration in muscle bundles and in the liver there was severe cirrhosis, necrosis and haemorrhage was observed. The study's findings are reviewed in light of the effects of paraquat dichloride on these freshwater fish.*

***Key Words:*** *Cirrhinus reba (Hamilton, 1822), Paraquat dichloride, biochemical and histopathology.*

## 1. INTRODUCTION:

Paraquat is a herbicide widely used to eradicate weeds which may impede the flow of aquatic life and create long-term environmental impacts. Repeated treatments of t are ineffective due to its poor persistence. Because of its low persistence, repeated applications of this herbicide are used for weed management in agricultural fields, resulting in significant amounts entering aquatic bodies. Chemicals like paraquat, originating from agricultural activity enter the aquatic environment. The toxicity of a chemical is entirely reliant on its concentration in organisms or even at the target receptor in the organism.

Paraquat (1, 1'-dimethyl, 4, 4'-bipyridinium dichloride) is an herbicide used in agriculture and aquatic habitats to control weeds. The application of paraquat in aquatic ecosystems may have detrimental consequences for aquatic creatures' health (Mahdi Banaee, 2016). Paraquat dichloride is a contact herbicide that becomes non-selective which used for the control of several broad leaved and grasses in plantations and other weeds in non-crop land/ urban and household settings worldwide (Aghoghovwia and Izah, 2018). When sprayed directly onto leaves, it quickly destroys a wide range of annual grasses, broad leaf plants, weeds, and some perennial grasses. Basically paraquat is a quaternary nitrogen based herbicides that destroy plant tissues. Furthermore, paraquat acts on the tissue of several plant through contact processes (Banaee *et al.*, 2013; Aghoghovwia and Izah, 2018). The effects of paraquat herbicides on human health are based on inhalation, ingestion, and damaged skin integrity (Arivu *et al.*, 2016). Ademola *et al.*, (2019) noted that, LC50 value of 40.7684 mg/L was also found to be the acute toxicity of paraquat dichloride to *Oreochromis niloticus* fingerlings. Bala *et al.*, (2019) reported that, an indication of subtle but rapid deterioration of life due to the effects atrazine exerted on fish was evident in the blood parameters because small sublethal concentrations of toxicants in water bodies is equally as harmful as the release of its acute concentrations. Mahdi Banaee *et al.*, (2016) supports research study's hypothesis because sub-lethal concentrations of paraquat had significant effects on the blood biochemical parameters of common carp. This indicates high mortality threshold. It is therefore concluded that this toxicity, Hence the usage must be monitored and controlled especially when in use close to any aquatic ecosystem. Herbicide residue damages key organs such as the kidney, liver, gills, stomach, brain, muscles, and organs. Aquatic bioassays are essential in water pollution control to review if a possible toxicant is harmful to aquatic life and, if so, to establish a link between toxicant concentration and aquatic animal effects. Ladipo, (2011) were noticed useful aspects on ecological and toxicological of paraquat.

Many reports are available on the effects of paraquat on fishes (Banaee *et al.*, 2013; Inyang *et al.*, 2016; Ojesanmi *et al.*, 2017). *Cirrhinus reba* (Hamilton, 1822), a bottom feeder omnivorous fish at the secondary level of the freshwater ecosystem, is widely cultivated and found in Indian fish farms. It is used as a biological indicator in ecotoxicological studies because it is a bottom feeder omnivorous fish at the secondary level of the freshwater ecosystem (Stegeman *et al.*, 1992). Therefore, the purpose of this study is to see if paraquat dichloride, at a sublethal dosage of

0.0029 ppm, has any influence on the biochemical and histomorphological alterations of the fish *Cirrhinus reba* (Hamilton, 1822) in chosen tissues such muscles and liver over 72 hours.

## 2. MATERIALS AND METHOD:

**Experimental Fish and Acclimatization**

The test fish *Cirrhinus reba* (Hamilton, 1822) were purchased from Mehrun lake of Jalgaon for the experiment. The present study was carried out during December 2019 to January 2020. The fish averaged 7.5±2 g and 5.5±2 cm in weight and length, respectively. The fingerlings were acclimatized for two weeks in a 25-litre rectangular plastic tank and fed pelleted feed. During acclimation, the water was changed regularly and the animals were fed twice a day.

**Experiment Design**

The experiment was set up in a completely randomised manner. At a stocking rate of ten fish per tank, a total of 150 selected experimental fish were randomly distributed into the aquarium (60 x 38 x 27 cm). The toxicity test was done as per the standard bioassay method of APHA (1991). A concurrent control having no death record was maintained in laboratory conditions. $LC_{50}$ and $LC_{10}$ values for 72 hrs were calculated by method of probit analysis (Finney, 1971) using commercial grades of the Paraquat dichloride (herbicide). A batch of ten fishes was exposed to desired sublethal concentration of Paraquat dichloride (herbicide) for 72 hours are 0.0029ppm.

**Biochemical Analysis**

Standard methods were used for biochemical investigations. glycogen was estimated following the Anthrone method of Van der Vier, (1954) as modified by Mahendru and Agarwal, (1982); For protein estimation by Lowry *et al.*, (1951); Lipid method by Folch *et al.*, (1957); acid phosphates ACP activity was assayed as described by Gutman and Gutman, (1940) and alkaline phosphates ALP activity was assayed as described by King, (1951). Each observation was confirmed by repeating at least for five times and difference in control and treated values statistically evaluated and significance using student‘t’ test (Bailey, 1965).

**Histological analysis**

At the end selected period of exposure the fish from the experimental groups were removed and dissected immediately for histological examination, whereas control specimens were vivisected without anaesthetic and subjected to histological analysis. Tissues were fixed in 4% formalin; they were embedded in paraffin, sectioned at 4 – 5 µm thickness, processed, block preparation, and cutting. Double stained / counter staining was done by using haematoxylin and eosin and examined using light microscope.

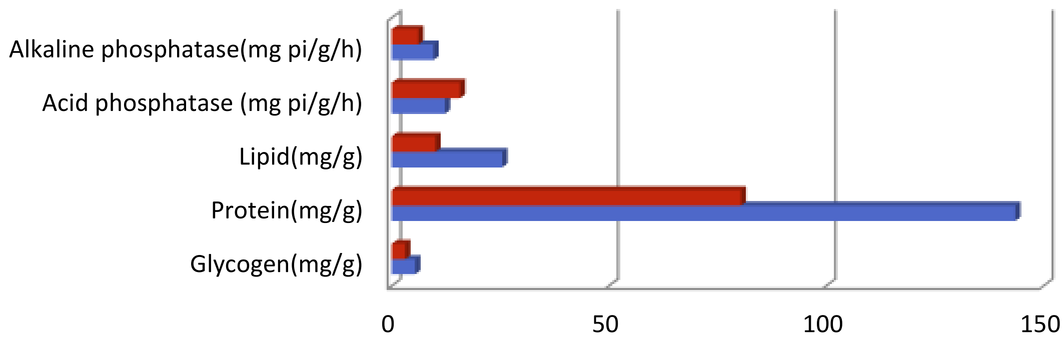
## 3. RESULTS AND DISCUSSION:

Graphs 1 and 2 summarised the findings. Biochemical changes for 72 hrs were significantly higher than in the respective control. Paraquat dichlorides are influence on biochemical parameters. Glycogen, protein, lipid, acid phosphates (ACP), and alkaline phosphates (ALP) levels in freshwater fish *Cirrhinus reba* (Hamilton, 1822) muscles and liver tissues. The glycogen, protein, lipid, and alkaline phosphates ALP contents of tissues depleted under herbicidal stress, whereas acid phosphates ACP content increased. However, the changes were not uniform in both tissues, and the physiological state of metabolic activity of an organism reflects in the utilisation of biochemical energy to counteract the toxic stress. Variation in biochemical and physiological changes show specific responses to certain type of environmental stressors such as herbicides.

Such reactions are especially valuable in fisheries management and resource conservation as a way of detecting potential environmental degradation. However, setting a standard as a diagnostic tool is still a work in progress (Christopher *et al.*, 2015). Paraquat's toxicological characteristics are linked to its capacity to create reactive oxygen species such as superoxide anion, which can cause cell death directly or indirectly (Sharifinasab *et al.*, 2016). The increase in the process of glycogenolysis in the fish tissues for immediate energy requirements to sustain the present stress might be linked to depletion of glycogen concentration (Rzymski *et al.*, 2013). Glycogen levels are depleted as a result of stress, during the detoxification process of active moieties and their metabolites. Tissue glycogen content has been studied by many workers in the fishes (Doherty *et al.*, 2011) exposed to herbicides. Depletion of liver protein suggests intense proteolysis, which leads to an increase in free amino acids fed into the TCA cycle as keto-acids and implies a fast breakdown of protein to fulfil energy demands during toxic stress, thus supporting the view of Nabela *et al.*, (2011). Lipids, like glycogen, are a type of energy storage. Lipids are an essential component of aquatic organisms, and measuring them can provide information about their development methods, health and condition, and survival capacity in both invertebrates and vertebrates (Arivu *et al.*, 2016). The concentration of lipids and glycogen related to liver failure as a result of toxicant exposure can be ascribed to portal and central venous congestions, cellular vacuolation, and infiltration. The activity of acid phosphatase was considerably increased in the gills and liver of stressed fish, possibly due to necrotic alterations in the organs of the body Aghoghovwia and Izah, (2018) have also suggested that the increase in lysosomal activity occurs as a part of prenecrotic changes. The strong toxic action of toxicant probably ruptures the cellular and lysosomal membrane that contains the hydrolytic enzymes, resulting in their increase. The alteration in the activity of acid phosphates enzyme after pesticides exposure might be due to damaged tissues of fishes may possibly have to secrete large amount of acid phosphatase. Alkaline phosphatase, (ALP) a brush border enzyme splits various phosphate esters at an alkaline pH and mediates membrane transport. Hence, decrease in alkaline phosphatase activity by the pesticide may result in altered transport and an inhibitory effect on the cell growth and proliferation (Akinsorotan *et al.*, 2019). Any damage in hepatic cells may results in alteration in ALP activity. Subsequently the enzyme activity may begin to alter either as a result of having partly or fully encountered the toxin or because of cell damage. The inhibition in protein level may also be due to the decrease in ALP activity as it plays an important role in protein synthesis. Toxic substances in the aquatic habitats are well known to cause various adverse effects on fish. Their accumulation in the aquatic organism could pose high risk of adverse health effect to the accumulating organisms. The biochemical changes in the organs of animal exposed to herbicides have no definite pattern and the physiological state of metabolic activity of an organism reflects in the utilization of their biochemical energy to counteract the toxic stress. A toxicants induce its effects at cellular or even at molecular level, but ultimately cause physiological, pathological and biochemical alterations.

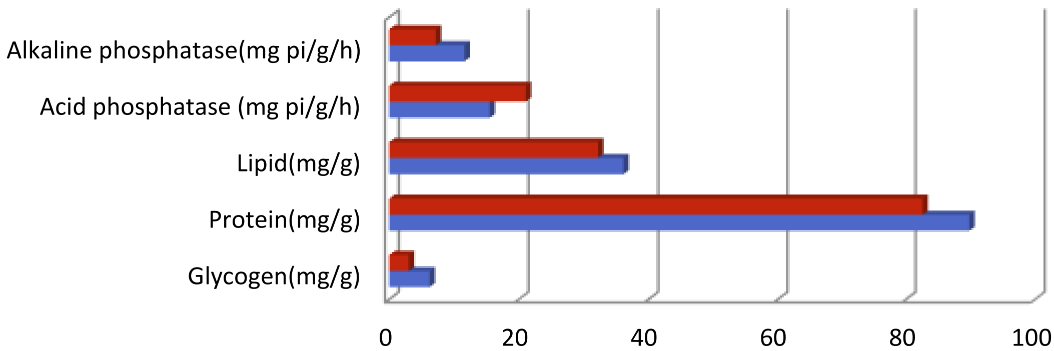
## 4. FINDINGS:

Graph 1. Biochemical parameters of muscles of *Cirrhinus reba* exposed to Paraquat dichloride.




| | Glycogen(mg/g) | Protein(mg/g) | Lipid(mg/g) | Acid phosphatase (mg pi/g/h) | Alkaline phosphatase(mg pi/g/h) |
|---|---|---|---|---|---|
| Exposed | 3.136 | 80.45 | 10.07 | 15.72 | 6.12 |
| Control | 5.423 | 143.5 | 25.51 | 12.25 | 9.62 |

Graph 2. Biochemical parameters of liver of *Cirrhinus reba* exposed to Paraquat dichloride.




| | Glycogen(mg/g) | Protein(mg/g) | Lipid(mg/g) | Acid phosphatase (mg pi/g/h) | Alkaline phosphatase(mg pi/g/h) |
|---|---|---|---|---|---|
| Exposed | 3.02 | 82.53 | 32.19 | 21.06 | 7.19 |
| Control | 6.223 | 89.8 | 36.1 | 15.48 | 11.61 |

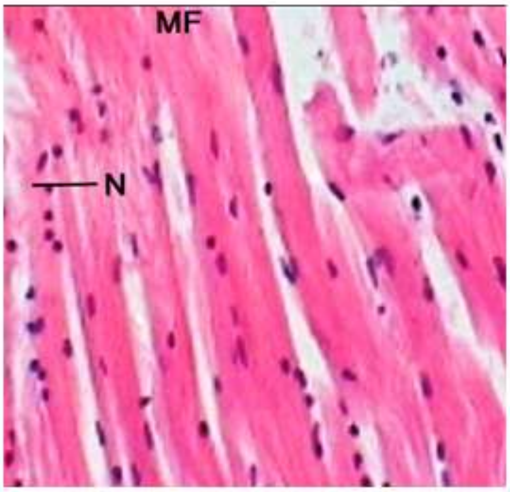



**Figure 1**

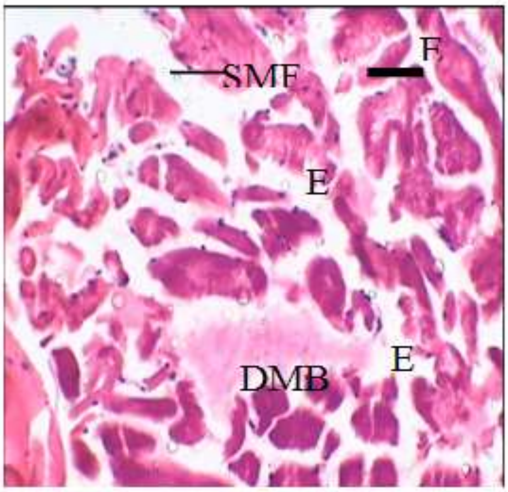



**Figure 2**

**Figure.1** T. S. of normal muscles of *Cirrhinus reba* , MF :Muscle Fiber; N : Nucleus; **Figure. 2** T. S. of gill of *Cirrhinus reba* exposed to sublethal concentration of paraquat dichloride for 72 h, H. ×400{SMF: Splitting of muscle fiber, E: Edema, DMB: Degeneration in muscle bundle} VDMB : Vacuolar degeneration in muscle bundle, AMP : Atrophy of muscle bundle.

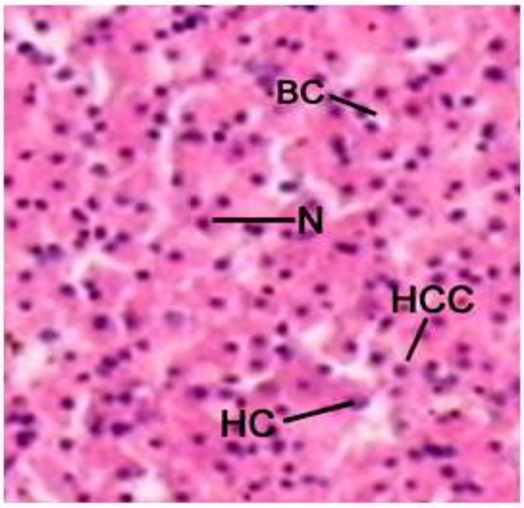



**Figure 3**

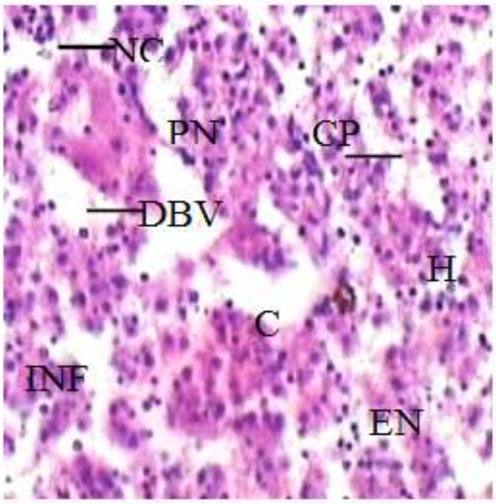



**Figure 4**

**Figure. 3** T. S. of normal liver of *Cirrhinus reba*, **Figure. 4** T. S. of liver of *Cirrhinus reba* exposed to sublethal concentration of paraquat dichloride for 96 h, H.E × 400.{ BC : Blood Capillary; N : Nucleus; HCC : Hepatic Cell Cord; HC : Hepatic Cells; EN : Enucleated; PN : Pyknotic Nucleus; CP : Cytoplasmic Pyknosis; H : Haemorrhage; NC : Necrosis; INF: Inflammation; C : Cirrhosis; DBV : Damage Blood Vessel}.

**Histological changes:**

**CONTROL MUSCLES (Fig. 1):** Normal histological structure of the striated muscles showed irregular vertical bands, and various patterns. These muscles compose the bulk of the body and are functional in swimming by producing body undulations that propel the fish forward. The muscle segments, called myomeres, are divided into an upper and a lower half by a groove running along the mid body of the fish. Fish muscles are divided into myotomes separated from each other by thin sheets or membranes made up of connective tissues (myocommata). Within each myotome, the muscle fibres run approximately parallel to each other, but at varying angles to the myocommata sheets to give the necessary moment for swimming during contraction. Fish muscle tissue consists of bundles of cylindrical multinucleated muscle cells. Each muscle cell, also called muscle fibre, is comprised of bundles of myofibrils arranged longitudinally within the muscle cell. In the periphery of the fish cell, the fibrils are elongated with, in cross-section a ribbon like shape. A fine network of collagen surrounds each muscle fibre and proceeds into the myocommata.

**Effect of Paraquat dichloride on Muscles (Fig.2):** The histopathological alterations the effects of paraquat dichloride in the muscles of the fish are noticed. Focal areas of myolysis were seen in the muscles. Moreover, destruction and vacuolation of the muscle cells were evident. The muscles showed degeneration in muscle bundles accompanied with necrosis (NC) as well as atrophy (AMB) and vacuolar (V) degeneration in muscle bundles. The severity of damage was more progressive in later stages of exposures.

**Control Liver (Fig. 3):** The liver of the control fishes showed no sign of any histimorphological damages throughout the period of study. The histology of control fish liver composed of parenchymal cells, lattice fiber, hepatic cells (HC) with central nuclei and granular cytoplasm, which support the former. Hepatic cells are roundish polygonal, containing clear spherical nucleus (N) venous blood enters the liver caudally from the hepatic portal veins and branches into capillaries known as sinusoids. Hepatic cells located among sinusoids forming cord like structures known as hepatic cells cord (HCC). In fish structure are generally obscure. Bile canaliculi (BC) are centrally located in each cord.

**Effect of Paraquat dichloride on Liver (Fig. 4):** These were more pronounced at 72 hours with sighs of extensive vacuolation (V) of hepatocytes and necrosis (N) in liver cells. They showed mild portal and central venous congestion after the 72 hrs. Several hepatocytes looked binucleated. Some pyknotic nuclei (PN), cytoplasmic pyknosis (CP) were also seen. Severe necrosis (NC), inflammation (INF), cirrhotic (C) was also observed. Blood vessel showed damage (DBV), haemorrhage (H) was also observed. They included vacuolar degeneration in the hepatocytes and haemolysis between the hepatocytes and dilation and intravascular haemolysis in hepatoportal blood vessels.

Histopathological abnormalities occur at the tissue and cellular levels are the result of complex physiological dysfunctions. Paraquat has been proven in studies to slow the development and weight gain of Oreochromis niloticus (Babatunde and Olajimeji ,2014), Histopathological changes which occur due to herbicidal impact on different organisms have been studied by several workers (Ogaga *et al.*, 2019). The mode of the action of the herbicide is species specific as the detoxification power is different in fish to encounter the toxic effects. Paraquat damages the liver and the kidneys, reduces O2 uptake by injuring gill epithelium, and can cause serious problems in the nervous system by inhibiting acetylcholinesterase enzyme activity. Many reports are available on the effects of paraquat on fishes (Rathod, 2011; Babatunde, and Oladimeji, 2014; Ojesanmi *et al.*, 2017; Ademola *et al.*, 2019). Elias *et al.*, (2020), results provide a description of serum biochemical and histopathological alterations in the liver and ovaries of the African catfish, C. gariepinus, that can be used as baseline information for further studies and suggest that C. gariepinus is a useful bioindicator animal for monitoring the effects of herbicide.

## 5. CONCLUSION:

The cumulative toxicological impact of herbicides encompassing their survival, disorders the metabolic activity. Herbicides altered the muscles and liver cytoarchitecture, which clearly reflected in its metabolic activity, according to physiology and histology. Depending on the herbicidal method of action, the degree of cellular damage varies.

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