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Impact of Chronic Paraquat Toxicity on Lipid Peroxidation Activity of Indian Major Carp *Cirrhinus Mrigala Ham.*

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Abstract Paraquat Dichloride, a broad-spectrum herbicide, used for the killing of aquatic weeds in aquaculture ponds and reservoirs was tested on extensively cultured Indian Major Carp (IMC) *Cirrhinus mrigala Ham.*. For this purpose, fingerlings of this economically important fish were exposed to the sub-lethal concentrations ($LC_{1/20th}$ and $LC_{1/10th}$) of paraquat continuously for 30 days by using the static renewal bioassay method. Post Exposure, the lipid peroxidation activity in 4 vital organs of the fish viz. gill, muscle, liver, and brain were estimated by E. D. Wills's method to rectify paraquat's toxicity. Annotated findings specified, a highly significant ($p<0.001$) chronological increase in lipid peroxidation activity (LPO) of gill, muscle, and liver tissues respectively in the $LC_{1/10th}$ concentration group, while a moderately significant ($p<0.01$) increase in LPO activity of brain tissue in the same group. While the gill and the brain tissue of the $LC_{1/20th}$ group showed moderately significant ($p<0.01$) and significant ($p<0.05$) increases in the LPO activity respectively. All changes showed their dependency on the time and concentration factor of the toxicant. Concluding the study, it was stated that, chronic paraquat sub-lethal exposure significantly increased the LPO activity in the vital tissues of *Cirrhinus mrigala Ham.* thus supporting its highly toxic nature and an immediate need to restrict its use as much as possible.

Keywords Paraquat Dichloride, Chronic Toxicity, *Cirrhinus Mrigala Ham.*, Lipid Peroxidation Activity

1. Introduction

Paraquat Dichloride ($C_{12}H_{14}Cl_2N_2$) 75-305-73-0 (CAS) is a quaternary nitrogen bipyridyl weedicide produced commonly in the form of brown color concentrated liquid consisting of 10-30% strong dichloride salt of it, sold under the brand name Gramoxone [1] by agrochemical company 'SYNGANTA'. In humans, paraquat ingestion causes hazardous effects in different organ systems. Such paraquat toxicity has no firm existing antidote therapy [2] [4]. The direct spray of paraquat on matured food crops to desiccate them for better marketability reasons is the current most alarming issue that has the highest chances of residual paraquat in the daily diet of humans and animals causing large-scale epidemic issues [5] [6]. Such extensive application of paraquat across various platforms of terrestrial and aquatic ecosystems has led to its far-reaching residues in soil and water that conclusively enter into the food chains [7]. Paraquat enters into aquatic ecosystems through surface run-offs, leaching, atmospheric deposition, drifting, etc., and accumulates into various organisms that reside in waters profoundly the fishes [8]. Numerous water bodies viz. Bois d'Orange River, Choc River, Cul-de-Sac River, Roseau Dam, Roseau River, Choc River, Cul-de-Sac

River, Soufriere River, Soufriere Dam, Cannelles River, have been found to contain paraquat [9]. The overall health and equilibrium of aquatic creatures may be negatively impacted by the direct application of paraquat to weeds in aquatic ecosystems [10]-[13] thus leading to severe acute as well as chronic toxicity in aquatic organisms. Fish's digestive system, skin, and gills may absorb paraquat from the residual surface waters [11] hence the study of its effects on fish is an important marker for the assessment of its eco-toxicity.

Pesticide poisoning has a key molecular mechanism called Lipid Peroxidation Activity (LPO) [14]. It is a detrimental attack of reactive oxygen species (ROS) that damages tissues and organs by oxidative stress [15]-[17]. Biota exposed to ambient pollutants may experience significant ROS activity, which may prevent the protective antioxidant system from eliminating them, resulting in oxidative stress and damage [18]-[21]. LPO is crucial for aquatic species since they have a much higher concentration of polyunsaturated fatty acids (PUFA) than terrestrial animals [22]. This study documented the changes in LPO activity as a consequence of malondialdehyde (MDA), a byproduct of PUFA peroxidation that occurs inside cells.

The present study was intended to investigate such toxic effects of paraquat dichloride on the lipid peroxidation activity in the fingerlings of commercially important Indian Major Carp *Cirrhinus mrigala Ham.*, as grassroots biomarkers of pesticide nuisance to the health of aquatic animals useful in the assessment of environmental risks.

2. Materials and Methods

2.1. Procurement and Rearing of Experimental Animal

The Government Fish Seed Production Center, Dhom (Wai), Satara District, Maharashtra State, India, supplied *Cirrhinus mrigala Ham.* fingerlings (mean weight- 3.18±0.21 gm. and mean length- 4.72±0.44 cm) for this study in large plastic bags sustained by oxygen. Fish were sanitized in a lab setting by being dipped for two minutes in a 0.1% $KmNO_4$ solution. After that, they were placed in well-aerated glass aquariums with regular dechlorinated faucet water, where fish underwent 15 days of acclimatization to room temperature. Standard procedures as illustrated in APHA [23] were used in the experiment to determine the physicochemical parameters of the water. Obtained values were as follows: Temperature 26.2 ℃, pH ranges 7.1-7.6, Dissolved oxygen (DO) content 5.61-6.13 mg/L, liberal CO_2 14.27 ± 0.47 mg/L, hardness 119.38 ± 3.72 mg/L, phosphate content 0.5 ± 0.03 mg/L, nitrates content 1.11 ± 0.26 mg/L. During acclimatization and experimental procedures, 2 percent fish food (Taiyo Discovery) of the fishes' average body weight was fed to them every day. Natural photoperiod was maintained. During the acclimatization, the aquarium water was changed every 24 hours to discard food remnants and fecal matter that can cause unnecessary stress in the enclosed water system. The water quality parameters were checked weekly to ensure normal conditions. Removal of any dead fish was done immediately to avoid possible water quality deterioration. After 15 days of acclimatization, the fish to be used for the experiment were screened critically for any indication of physical damage, disease, stress, and mortality. Any suspected fishes were discarded immediately and only the healthy fishes were selected for the study. Before initiation of experimental protocols, the fish were acclimatized to well-aerated 22-liter capacity plastic containers for 7 days, during which they were to be exposed to the toxicant. 24 hours before the test, feeding was discontinued to reduce the effect of vomiting and excess animal excreta due to the toxicant, but later, after 24 hours of exposure, the feeding was restored at 2% of their body weight.

2.2. Exposure to Paraquat

The herbicide paraquat dichloride commercially sold under the brand name Gromoxone (24%w/w) by Syngenta was used as a toxicant in the present study. Before the experiment, a study was conducted to determine the mortality rate of fishes exposed to various concentrations of paraquat by the static renewal bioassay method. The data so obtained was processed by Finney's Probit analysis to obtain the LC_{50} (105 ppm) value for the toxicant used. Both the sub-lethal concentrations viz. $LC_{1/20th}$ and $LC_{1/10th}$ concentrations used in this study were derived from this LC_{50} value. For the current experiment, three clear, open-mouthed, cylindrical plastic jars with a volume of 22 liters were arranged side by side in a row. To sustain the desired level of dissolved oxygen present in the water, each jar received continuous, appropriate aeration. All containers were filled with 20 liters of clean dechlorinated tap water and 10 fish were released in each of them. The fish in the first jar functioned as a control group because they weren't subjected to any toxicant. The fingerlings in the second and the third jars were subjected to paraquat doses of $LC_{1/20th}$ (pre-calculated: 5.25 ppm) and $LC_{1/10th}$ (pre-calculated: 10.5 ppm) for 30 days each (chronic toxicity). At every 24 hours, the water medium and the toxicant in all the containers were replaced with fresh water and toxicant, to maintain the optimum concentrations throughout the experiment. Any dead fish if observed were removed immediately from the container and buried underground in follow land away from domestic areas. Live juvenile fingerlings from all three jars were euthanized after 30 days of exposure, and the LPO activity in their gills, livers, muscles, and brains was examined in each organ separately. The remains of the euthanized fish too were buried underground in same area where dead fish were buried.

2.3. Analysis of the Lipid Peroxidation Activity (LPO)

In the current investigation, the changed levels of lipid peroxidation in all 4 tissues were estimated using E. D.

Wills's protocol [24]. The reaction mixture necessary to homogenize the tissues was presumably prepared fresh. To make the reaction mixture, 1 ml of Phosphate Buffer Saline (PBS, pH 7.4), 1 ml of 1 mM $FeCl_3$, 0.01 ml of Chlorotetracycline, and 1 ml of 75 mM Ascorbic Acid (AA) were blended together. Following that, 10 ml of this reaction mixture was used to homogenize 100 mg of fresh tissue. This mix was used as a stock. Following that, 1 ml of this stock solution was divided into three test tubes (triplicates), and 1 ml of distilled water, 1 ml of 20% TCA, and 2 ml of 0.67% thiobarbituric acid (TBA) were all added to each of these triplicates. In an additional test tube, a blank was simultaneously made by mixing 2 ml of distilled water with 1 ml of 20% TCA and 2 ml of 0.67% TBA. Following that, for 15 minutes, all test tubes were submerged in a bath of boiling water. The test tubes were cooled for 15 minutes and centrifuged at 1000 g for 10 minutes. Then, using a spectrophotometer, the absorbance readings of the supernatants so acquired were measured against the blank at 532 λmax. Using 1.56 x 105 M^{-1} cm^{-1} as its molar extension coefficient, the lipid peroxidation levels were estimated as nano-moles (nM) of MDA generated per milligram (mg) of tissue (nmol MDA/mg tissue). The following formula was used to determine the level of MDA.

$$\text{MDA / mg tissue} = \frac{\text{O.D. of the sample}}{(0.156)\,(1)}$$

Where,

0.156 = 1 mM Malondialdehyde solution's absorbance at 532 nm in a cell that is 1 cm thick.

1 = Amount of tissue taken in mg, present in 1 ml of a sample.

The final data from all the groups was expressed in Arithmetic Mean (AM) ± Standard Deviation (SD) format. Utilizing the unequal variance (2-sample) (heteroscedastic) approach of "student's T-Test" with "two-tailed distribution", the significance level was determined. If $p < 0.05$ it means it showed a significant change. If $p < 0.01$ it showed a moderately significant change and, if $p<0.001$ it showed highly significant change.

3. Results

The readings representing the effects of paraquat poisoning on the lipid peroxidation activity of *Cirrhinus mrigala Ham.* gills, brain, muscle tissue, and liver in the control group, $LC_{1/20th}$ concentration set, and $LC_{1/10th}$ concentration group after chronic exposure (30 days) are represented in Table 1. In the control group fish *Cirrhinus mrigala Ham.*, the LPO activity was found to follow the Brain > Liver > Muscle > Gills sequence.

The lipid peroxidation activity in gill tissue showed a moderately significant ($p<0.01$) increase in the $LC_{1/20th}$ group (t.stat = -7.9009) while it showed a highly significant ($p<0.001$) increase in the $LC_{1/10th}$ group (t.stat = -17.901). The lipid peroxidation activity in muscle tissue showed a highly significant ($p<0.001$) increase in the $LC_{1/10th}$ group (t.stat = -16.1532). The lipid peroxidation activity in liver tissue showed a significant ($p<0.05$) increase in the $LC_{1/20th}$ group (t.stat = -3.6360) while in the $LC_{1/10th}$ group (t.stat = -13.026) it showed a highly significant ($p<0.001$) increase. The lipid peroxidation activity in brain tissue showed a significant ($p<0.05$) increase in the $LC_{1/20th}$ group (t.stat = -3.5901) while it showed a moderately significant ($p<0.01$) increase in the $LC_{1/10th}$ group (t.stat = -4.884). The post-experimental lipid peroxidation activity in the four tested tissues was in the order Liver > Brain> Gill > Muscle in the $LC_{1/20th}$ group while in the $LC_{1/10th}$ group it was in the order, Brain > Liver > Gill > Muscle.

Table 1. Effect of Paraquat Dichloride on the Lipid Peroxidation activity in different tissues of the fish *Cirrhinus mrigala Ham.* after chronic exposure

Groups	Lipid peroxidation activity (nM of MDA/mg wet wt. of tissue)			
	Gill	Muscle	Liver	Brain
Control Group	1.76 ±0.14	1.92 ±0.14	4.98 ±0.12	5.12 ±0.33
$LC_{1/20th}$	3.15 ±0.26 **	3.03 ±0.72	6.01 ±0.47 *	5.9 ±0.16 *
$LC_{1/10th}$	4.84 ±0.26 ***	4.6 ±0.24 ***	6.8 ±0.2 ***	7.43 ±0.74 **

(Values in table no 1 are expressed as Arithmetic Mean of (n= 6); ±SD), *= p<0.05 (significant), **= p<0.01 (moderately significant), ***= p<0.001 (highly significant)

The results of the current study illustrate the significance levels of MDA activity that are directly proportional to LPO activity in vital tissues of fish after exposure to paraquat. $LC_{1/10th}$ group show highly significant MDA activity in comparison to control group while $LC_{1/20th}$ group shows a just significant impact on MDA activity as compared to control. Thus the higher concentration ($LC_{1/10th}$) of paraquat tends to make a highly significant impact on LPO activity in vital tissues of exposed fishes as compared to the lower concentration ($LC_{1/20th}$) of paraquat.

4. Discussion

Lipid peroxidation results from oxidative injury driven by ROS action that disrupts cell anatomy and physiology [25] [26]. Fish are useful markers of pollutants that allow for early detection of aquatic issues related to environmental health [27] [28]. Internal cellular constituents of fish are harmed when toxicants promote the aberrant creation of ROS and it surpasses the intrinsic defense system of the fish. The term "oxidative stress" refers to this phenomenon [29]. Pesticide impacts causing oxidative stress had been widely investigated as a potential mechanism illustrating their toxicity and

degradability, in a variety of tissues [30]. Malondialdehyde (MDA) is a byproduct of LPO. MDA synthesis is a crucial sign of induced oxidative stress driven by free radicals that harm biological membrane constituents [31]. The findings of the current study reveal noticeably higher rates of MDA activity in all four tissues, i.e. the gill, muscle, liver, and brain subjected to Paraquat at both $LC_{1/20th}$ and $LC_{1/10th}$ concentrations. These elevated levels of MDA are a sign of the peroxidation of lipids set on by Paraquat poisoning in key fish tissues. The harmful effects of Paraquat may have resulted in the oxidation and redox-cycling of cellular NADPH that potentially releases a large amount of ROS, which ultimately led to oxidative stress and damaged essential tissues via lipid peroxidation. The generated malondialdehyde also reacts actively with other biomolecules like proteins, changing their structures, properties and functions [32]. Lipid peroxidation reduces the nutritional value of edible fish meat, creating health issues as well as the monetary loss for the stakeholders [33]. Persch et al. [34] showed similar results, that *Rhamdia quelen* fingerlings exposed to multiple paddy herbicides used in integrated rice-fish farming demonstrate a comparable rise in lipid peroxidation rate in their gill, liver, kidney, and muscle tissues. Similarly, [35] studied cadmium induced physiological alterations in Nile tilapia and reported increased lipid peroxidation activity with elevated levels of toxicant as compared to control group.

5. Conclusions

The present study's findings suggest that paraquat poisoning can have a significant detrimental effect on non-targeted creatures like fish because it elevates the levels of lipid peroxidation activity in their vital organs. Increased LPO activity has the potential to impair fish's ability to operate optimally and maintain its internal homeostasis, which might result in fish death, growth retardation, and poorer-quality fish meat, which might harm the stakeholders' nutritional needs as well as their health and exacerbate aquatic pollution. LPO activity can also function as a reliable biomarker of how anthropogenic stresses affect unintended organisms.

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