Paraquat Toxicity on Selected Biomarkers in *Clarias gariepinus*

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**Abstract:** Pesticides are major sources of environmental pollutants utilized to control or eradicate pests negatively impacting on man agricultural resources. Amid the varied classes of pesticides, Paraquat dichloride, an organochloride is widely deployed against varieties of agricultural and domestic pest with potential consequences. This study examined the impact of Paraquat (1,1-dimethyl 4, 4-bipyridiniumdichloride) on the gills and liver of *Clarias gariepinus*. In the acute studies, fish were allocated into five groups and exposed to 50, 100, 150,250 and 250 mg L of Paraquat for 96 h. In chronic exposure, fish were distributed into 2 groups and exposed to 1/10 and 1/100 96 Hr LC50 value of (128.03 mg L) of Paraquat for 30 days. Liver and gills were harvested and evaluated for antioxidant parameters and histopathology respectively. There was no significant induction (P > 0.05) in the activity of SOD in exposed organisms while CAT enzyme activity was significantly (P < 0.05) induced in exposed organisms when compared to the control group respectively. GSH induction (P > 0.05) in the activity of SOD activity in exposed organisms while CAT enzyme activity was significantly (P<0.05) inhibited , while MDA was significantly (P<0.05) induced in the exposed organisms throughout the duration of the study. Histology of the gills revealed increasing incidence of lesion, disorganization of the whole length of the cartilaginous support of the primary lamellae, hypotrophy of the secondary lamellae, lamellae fusion and blood clot with increasing duration of the study. The histological alterations in the gill tissue suggest they were induced by increased concentrations of the test chemical. Therefore, combined antioxidant parameters and histopathological studies are imperative for the assessment and evaluation of potential pollutants in the aquatic environment.

I. Introduction

Pesticides comprise of heterogeneous groups of chemical products basically used to stave off, control or eradicate pests negatively impacting on man agricultural resources. Ultimately, man has derived great benefits by the increased yield from agricultural products pesticide usage offers (Cavalcante, Martínez & Sofia 2008; Atamanalp, et. al., 2001). Despite its role in crop yield boost, pesticides have been reported to evoke negative consequences on the ecosystem. Pesticides find their way into the environment, either through water runoff and or as aerosols carried by winds from the indiscriminate application of pesticides in the field or from reckless storage and disposal of pesticide containers. The regular incursion of farmland effluents into fresh water sources often leads to diverse contamination incidences which become significant when co-occurring to other sources of pollution (Richardson, 1988).

High levels of pesticide contamination in aquatic ecosystems results into poisoning, oxygen depletion and resultant mass mortality of aqua-fauna (Atamanalp, et. al., 2001; Seiyaboh et al., 2013).

Among all classes of pesticides, Herbicides account for the gross use, symbolizing over 80 percent of pesticides utilized in farmlands globally (Gerald et al., 1995). Herbicides have the potential to accumulate in water bodies to levels detrimental to the survival of zooplankton which are main source of food for small and young fishes (Doherty et al., 2011).

Paraquat (1,1-dimethyl-4,4-bipyridinium) is one of the most broadly used herbicidal product globally and is currently accessible in more than 130 countries (Kimbrough 1974; Calderbank 1975; Data 1978; Haley 1979; Hughes 1988; Suntres, 2002 ; Wang et al., 2012). It is a principal element of the bipyridylum family of non-selective herbicide developed first commercialized for agricultural use in the United Kingdom in 1962 (Chia et al., 1982). Paraquat readily disintegrates and can persist in the environment absorbed to soil particles (Seiyaboh et al., 2013). Even at minor levels of concentrations, it still could elicit deleterious effects, such as cytogenetic damage, physiological effects, and even death to exposed non-target species ( Dinis-oliveira et al., 2008). Studies have shown that Paraquat has the potential to impede the growth and weight of *Oreochromis niloticus* (Babatunde and Olajimeji, 2014), negatively impact on the blood plasma activities of *Clarias gariepinus* (Seiyaboh et al., 2013), induce Respiratory stress, erratic swimming and instant death of fish.
Paraquat Toxicity on Selected Biomarkers in *Clarias gariepinus*

(Doherty *et al.*, 2011) impair the physiological processes in *Clarias gariepinus* by significantly increasing the level of white blood cells, glucose, aspartate aminotransferase, and alanine aminotransferase (Nwani *et al.*, 2015), impacts on the immune and growth of the rainbow trout, *Oncorhynchus mykiss* (Amir *et al.*, 2014). At high levels, Paraquat inhibits the photosynthetic ability of some algae in stream water thus disrupting the food web necessary for ecological balance (Kenneth, 1990).

The use of bioassay techniques to evaluate the toxicity of chemicals on animals has been used as suitable models to predict hazards posed by chemicals to man. Aquatic bioassay most especially is very crucial in water pollution management to verify if a potential toxicant is perilous to aquatic fauna and if so, to establish the relationship between the toxicant concentration and its effect on aquatic life (Olaila *et al.*, 2003).

Histopathological deformities can be used as biomarkers of the effects of anthropogenic pollutants on organisms and are suitable as indicators of the overall ecosystem health (Velkova-Jordanoska and Kostoski, 2005). These histological distortions are intimately linked to diverse biomarkers of stress because pollutants usually undergo metabolic activation in order to induce cellular modifications in the affected organism. Biochemical parameters have also been deployed to assess environmental burdens in response to prevailing pollutants; these parameters include the levels of plasma proteins, glucose, and other enzymes, like alanine aminotransferase (ALT), superoxide dismutase (SOD), catalase (CAT), glutathione S-transferases, glutathione, lipid peroxidation (MDA), and aspartate aminotransferase (AST) (El Sayed *et al.*, 2007; Suvetha *et al.*, 2010).

However, studies on the chronic impacts of Paraquat on tropical fish have been largely insufficient. The African catfish (*Clarias gariepinus*) is a major source of animal protein in Nigeria due to its relative abundance in natural fresh water bodies, inexpensive cost, and ease of rearing them in local ponds. This fish species can easily be acclimatized to laboratory condition, thus, provides an excellent model for ecotoxicological studies. The aim of the present study is to evaluate the sublethal effects of Paraquat on the histology and biochemical parameters in *C. gariepinus*.

### II. Materials And Methods

2.1. **Experimental fish and chemical**

Juvenile African catfish (*C. gariepinus*) of average weight and total length of 52 g and 17 cm, respectively was collected from a fish farm located in Agege local government area, Lagos state and transported to the laboratory where they were acclimatized for 7 days in a plastic tank (36 x 30 x 48.5 cm). The water used for stocking the test organisms in the laboratory was adequately dechlorinated by aerating tap water in a plastic container with the aid of an aerator (Cosmo Aquarium, air pump 11,000) for 27 hours. This was done to enhance swift vaporization of chlorine gas in the water. The fingerlings were fed with Coppens fish feed throughout their acclimatization. The organism were fed twice daily at 12 hour intervals (morning and evening) and the holding water was changed once every two days to avoid the accumulation of food residue and waste metabolite.

Feeding was terminated 24 hours before the commencement of the experiment as recommended by Ward and Parrish (1982), Reish and Oshida (1987). The trademark name of the Paraquat used in this study was Paraforce manufactured by Red Sun Group Corporation, China.

2.2. **Measurement of Physico-Chemical Characteristics of Test Media**

Measurement of the Physico-chemical characteristics measurements were taken at the inception of the experiment and at the end (that is, before change of test media). The parameters recorded are dissolved oxygen, pH, total dissolved solids, conductivity and salinity using appropriate digital instruments (Jenway).

2.3. **Acute toxicity testing**

96 h LC50 value acute bioassay were conducted in 25 L glass tanks (36.5 x 25 x 28 cm) in a static laboratory system with the test solution kept constant throughout the duration of the study. A set of 10 fish per three replicates were randomly exposed to the test chemicals at 50, 100, 150, 200, 250 mg/l respectively and an untreated control. The quantal response (mortality) was assessed every 24 hours over a period of 96 hours. Mortalities were recorded when they showed no response to mechanical stimulation when prodded with a glass rod. Dead specimens were removed to avoid pollution of the water. During the experiment, fish behavior was observed for opercula count.

2.4. **Sublethal effects of paraquat on Clarias gariepinus**

In the course of the experiment test organisms were exposed to sublethal concentrations (1/10th and 1/100th of 96h LC50) of the test compound extrapolated from the acute toxicity bioassay. A semi static bioassay test protocol was utilized in which the test media were changed once every 24 hours to fresh media of the same concentration and untreated control. At the end of the experimental procedures on day 28, test
organisms were retrieved, sacrificed and dissected to obtain gill tissues required for histological studies and biochemical assays and.

2.5 Preparation of Tissue Homogenates
At a pre-determined day, gills of test samples were harvested from sacrificed organisms. They were washed free of all blood residues in ice cold isolation medium (0.25M sucrose, 5mM tris HCL), lightly blotted and weighed. The gills were cut into fragments and homogenized (9% W/V) in 100% methanol and centrifuged at 10,000xg for 15min at 40C after method described by Hermes-Lima et al.,(1995). The supernatant was collected for assays.

2.6 Enzymes Activity Assays
Superoxide Dismutase (SOD): SOD enzyme activity was analyzed using the method as described by Sun and Zigman (1978). The SOD enzyme assay measured the difference between superoxide anion disintegration and synthesis i.e, its ability to repress the autoxidation of epinephrine. Enzyme action was observed at absorbance level of 450nm. Concentrations are presented as U/mg or SOD-Unit/mg protein, where one unit is expressed as the level of enzyme required to inhibit 50% epinephrine reduction per minute and per milligram of protein at 25 °C and pH 7.8.

Catalase (CAT): Catalase activity was analyzed following the protocol adopted by Cohen et al., (1970). The protocol focuses on measuring the rate of H2O2 disintegration at absorbance levels of 240nm. The results were presented as U/mg or CAT-units/mg protein, where one unit is the level of enzyme that hydrolyzes 1 µmol of H2O2 per minute and per milligram of protein at 30°C and pH 8.0.

Glutathione-S-Transferase (GST): The level of GST activity was analyzed according to the protocol adopted by Habig and Jakoby, (1981). The measurement of GST activity was carried out by monitoring at absorbance level of 340nm, the induction of a conjugate between 1mM GSH and 1mM 1-chloro-2, 4-dinitrobenzene (CDBN). The results were presented as U/mg or GST unit/mg protein, where one unit is expressed as the amount of enzyme that conjugates 1 µmol of CDBN per minute and per milligram of proteins at 25°C and pH 7.4.

2.7 Lipid Peroxidation (LPO) Assay
The levels of homogenized tissue malondialdehyde (MDA), as an index of lipid peroxidation were analyzed by thiobarbituric acid reaction (TBARS Assay) using the protocol adopted by Yagi (1998). In this method, malondialdehyde is evaluated spectrophotometrically at absorbance levels of 535nm to assay for the amount of lipid peroxidation in a sample.

2.8 Histopathology studies
Test specimen organs were extracted and processed for histopathological review. They were fixed in bouin’s fluid for 24 hours, rinsed with 70 percent ethanol and dehydrated through a graded series of ethanol (Schalm et al, 1975, Kelly, 1979). They were embedded in paraffin, sectioned at 4-5 um thickness stained with haematoxylin and eosin and examined using light microscope and photomicrography (Keneko, 1989).

2.9 Statistical Analysis
Acute toxicity data involving quantal response (mortality) were analyzed using the probit analysis after Finney (1971). The enzyme activity and lipid peroxidation assessment data were subjected to a one way analysis of variance (ANOVA) using graphpad prism 7 statistical package to compare means and to determine the significance differences at a 5% probability level.

III. Results

3.1 Acute Toxicity
Composed 96hours LC50 for Paraquat exposed to fish was 128mg/L(Table 1).

<table>
<thead>
<tr>
<th>PARAQUAT</th>
<th>LC50 (95% CL) % (mg/L)</th>
<th>SLOPE + S.E</th>
<th>d.f</th>
<th>PROBIT EQUATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paraquat</td>
<td>128.124 (172.827– 89.506)</td>
<td>4.940 ± 1.575</td>
<td>2</td>
<td>y = 3.75 * x + -7.5</td>
</tr>
</tbody>
</table>

KEY: cl = Confidence limit

df = Degree of freedom

DOI: 10.9790/2402-1205016675 www.iosrjournals.org 68 | Page
3.2 Biochemical Analysis

3.2.1 Superoxide dismutase (SOD)
The activity of the enzyme SOD in all exposed groups was inhibited (P > 0.05) on day 14 (1.91 - 2.02 µ/mg) and induced (P > 0.05) on day 28 (1.79 - 2.35 µ/mg) when compared to day 0 (2.13 µ/mg). There was no significant difference (P > 0.05) in the activity of SOD on day 14 and day 28 when compared with each other (Figure 1).

3.2.2 Catalase (CAT):
The activity of the enzyme CAT in exposed fish was induced (P > 0.05) on day 14 (9.25-10.1 µ/mg) and day 28 (7.73-11.1 µ/mg) when compared to day 0 (2.4 µ/mg). The induction of the enzyme of CAT on day 28 was significant (P<0.05) when compared to day 0 and day 14 respectively (Figure 1).

3.2.3 Reduced Glutathione transferase (GSH)
The enzyme activity of GSH was significantly inhibited (P < 0.05) in exposed groups on day 14 (0.47- 0.53 µ/mg) and day 28 (0.34-0.58 µ/mg) when compared to day 0 (0.72µ/mg). Furthermore, there was a significant difference (P<0.05) in the inhibition of GSH activity between sublethal concentrations on day 14 and 28 when compared to each other (Figure 1).

3.2.4 Lipid Peroxidation (LPO) Assay
The activity of the enzyme MDA was significantly induced (P < 0.05) in exposed fish on day 14 (0.083-0.093µ/mg) and day 28 (0.082-0.093µ/mg) when compared to day 0 (0.034) (Figure 1).

3.2.5 Glutathione S-Transferase (GST):
The activity of the enzyme GST was inhibited in exposed fish on day 14 (0.57-0.61µ/mg) and day 28 (0.54-0.61µ/mg) when compared to day 0 (0.64). GST activity on day 14 and day 28 in all exposed groups was not significant (P > 0.05) when compared with each other and with day 0 (Figure 1).

![Figure 1 Biochemical parameters of C. gariepinus exposed to sublethal concentration of Paraquat.](image)

3.3 Histology
Sections through the gill at day 0 revealed a pristine state of the entire length of the extracellular cartilaginous support of the primary lamellae (B), long primary lamellae (C) and long comb-like secondary lamellae (E) projecting from both sides of each primary lamella, epithelium lining on the membrane (D) and chondrocyte cells (A) (Fig. 2). There was no observable gill defects such as blood congestion, hyperplasia of the gill epithelium, lesion, epithelial lifting of lamellae, lamellar fusion, lamellar disorganization and hypertrophy of the gill epithelium (Fig. 2). At day 14, the histologic section of the gill filament revealed a mild degree of lesion (A), disorganization of the whole length of the cartilaginous support of the primary lamellae (B), hypotrophy of the secondary lamellae (C), and incidence of lamellae fusion (D) (Figure 3). At day 28, exposed gills of fish to the test chemical showed a severe degree of lesion (E), lamellae disorganisation and blood congestion (F) (Figure 4).
Paraquat Toxicity on Selected Biomarkers in Clarias gariepinus

Figure 2: Showing a pristine state of the entire length of the extracellular cartilaginous support of the primary lamellae (B), long primary lamellae (C) and long comb-like secondary lamellae (E) projecting from both sides of each primary lamella, epithelium lining on the membrane (D) and chondrocyte cells (A).

Figure 3: Day 14 exposed gills to toxicants revealing mild degree of lesion (A), disorganization of the whole length of the cartilaginous support of the primary lamellae (B), hypotrophy of the secondary lamellae (C), and incidence of lamellae fusion (D).
**IV. Discussion**

In ecotoxicological studies, the 96 h LC₅₀ is one of the most essential parameters for evaluating the toxicity of pollutants. Herein, the 96 h LC₅₀ value (i.e. 128 mg/L) obtained from *C. gariepinus* exposed to Paraquat, suggests that this herbicide is moderately toxic to fish. This is in contrast to previous studies of Paraquat exposed to *C. gariepinus* (27.46 mg/L) (Nwani et al., 2015); *Mesopotamichthys sharpeyi* (1.48 mg/L) (Safahieh et al., 2012); *Trichogaster trichopterus* (1.41 mg/L) (Banaee et al., 2012); and *Oreochromis niloticus* (7.00 mg/L) (Ada et al., 2012). Pesticide toxicity on an organism is affected by age, sex, size, species strain, water quality, species strain, formulation of test chemical and temperature (Pandey et al., 2005; Nwani et al., 2013).

Antioxidant enzymes such as SOD, CAT, GSH, MDA and GST perform a crucial role in the adaption of organisms to environmental stress through the riddance of Reactive oxygen species (ROS) (Barata et al., 2005; Zelikoff et al., 1996). The first line of defense commences with the activity of the enzyme SOD which converts hydroxyl ion(O₂⁻) into oxygen(O₂) and hydrogen peroxide(H₂O₂) (Singh et al., 2006). H₂O₂ is further detoxified by other enzymes such as CAT. CAT is located in the peroxisomes, cytosol and mitochondria and is capable of scavenging H₂O₂ into H₂O and O₂ respectively (Zhang et al., 2007a).

In the present study, the activity of SOD in fish was triggered with increasing exposure to Paraquat. This reaction is linked to the generation of excess ROS which activated the biosynthesis of SOD, better suited to shield the cells against oxidant damage (Zhang et al., 2004a; Zhang et al., 2013). This aligns with Oruc & Usta (2007) who described an increase of SOD content, in the gill and muscle of *Cyprinus carpio* after diazinon exposure. This increase in the SOD activity indicates the increase in O₂⁻ production (Zhang et al., 2004a).

CAT is responsible for the breakdown of H₂O₂ to water and oxygen, protecting the cell from further damaging action of H₂O₂ and the hydroxyl radical. The significant induction (P < 0.05) in the activity of CAT after 28 days mirrors the high H₂O₂ production from the activity of SOD and the increased oxygen consumption (Ritola et al., 2002a). Similar findings have been reported by Atif et al., (2005); Bouraoui et al., (2008); Dabas et al., (2012).

GSH is an endogenous antioxidant, which counters cellular components impairment hyperoxides and ROS (Pompella, 2003). Despite it activity as an express free-radical hunter, GSH also acts as a substrate for GST. The significant decline in GSH level may be attributed to its deployment to arrest the invasive ROS generated from Paraquat oxidative trigger. Decline in the GSH level has already been observed in fresh water fish exposed to other organophosphate methyl parathion (Monteiro, 2006).

GST is a microsomal enzyme that neutralizes a vast range of endogenous metabolic by-products and xenobiotics via enzymatic glutathione conjugation, glutathione-dependent peroxidase activity or isomerisation reactions (Hayes et al., 2005). Therefore, it also performs a valuable role in safeguarding tissues from oxidative stress (Fournier et al., 1992; Jiľa et al., 2006). The enzyme GST has been reported to exhibit varying response to different compounds, for example, inhibition of the enzyme was reported in animals exposed to lead and zinc (Awoyemi et al., 2014), dimethoate (Hamed et al., 1999), Trichlorfon (Thomaz et al., 2009). In contrast, statistically significant induction in GST was reported in organisms exposed to oxidative stress of 2,4-D.
Paraquat Toxicity on Selected Biomarkers in *Clarias gariepinus*

dichlorophenol (Zhang et al., 2004); Methylmercury (Neto et al., 2008); Endosulfan (Kono & Fridovich, 1982) and Carbaryl (Ferrari et al., 2007). In this study, the activity of GST was reduced in the examined liver of fish tissues exposed to Paraquat when compared to control. This result aligns to the finding by Saliu and Bawa-Allah (2012), who reported a decrease in the level of GST-GSH activity in the liver of post-juvenile *C. gariepinus* exposed to Pb(NO₃)₂ after 30 days. The decrease in GSH level and GST activity was caused by the ROS overproduction which depleted GSH and inactivated GST (Farombi et al., 2008).

The significant increase in MDA is a pointer of intense oxidative stress (Buyukokuroglu et al., 2002). This observation is in agreement with a study by Vadha and Hasan, (1986), who reported a significant increase in MDA content in *Heteropneustes fossilis* exposed to Dichlorvos. Similar elevation of MDA level was reported in *Cyprinus carpio* and *Ictalurus nebulosus* exposed to Dichlorvos (Hai et al., 1997). Oruc, (2011) also reported similar findings in Diazinon exposure in the liver of *C. carpio*.

The gills of fish have a thin, expansive surface directly in contact with the aquatic medium, thus making the organ an entry point for numerous environmental stimulants (Haaparanta et al., 1997; Baskar; 2014; Poleksic and Mitrovic - Tutundzic, 1994). Furthermore, the gills is the principal site for responding to unfavourable environmental conditions (Benli and Ozkul 2008). These peculiarities imply that the gill epithelium is highly exposed to environmental xenobiotics. In this study, the severity of lesions with increasing duration suggests a reflection of the direct reaction of the toxic compound on the tissue (Temmink et al., 1983; Marina and Claudia, 2007). Lesions impacts on the gill morphology resulting in the disruption of basic physiological processes, such as antioxidant defence or osmoregulatory mechanisms (Yancheva et al., 2015). Deformities such as hypertrophy of the epithelial cells, fusion of some secondary lamellae may indicate trigger of defense mechanisms, since, in general, these culminates into an increase of the space between the external medium and the blood. Thus, serving as a barrier to contaminant interactions (Mallatt, 1985; Hinton & Lauer, 1990; Poleksic & Mitrovic-Tutundzic, 1994; Fernandes & Mazon, 2003). The aftermath of the increased distance between water and blood due to the deformities is the impairment of oxygen uptake. Fishes are capable of countering this effect by increasing their ventilation rate, to compensate low oxygen uptake (Fernandes & Mazon, 2003). The gills alterations are nonspecific and can be triggered by different groups of toxicants (Mallatt, 1985). Similar gills deformities have also been reported in the fishes exposed to organic contaminants (Rosety-Rodriguez et al., 2002; Fanta et al., 2003) and metals (Oliveira Ribeiro et al., 2000; Cerqueira & Fernandes, 2002; Martinez et al., 2004).

These gills distortions occur very early in fish than physical and behavioral changes (Yancheva et al., 2015). Thus, they are useful as effective biomarker for estimating the impact of extensive herbicide application on *Clarias gariepinus*.

**References**


DOI: 10.9790/2402-1205016675 www.irosjournals.org 72 | Page
Paraquat Toxicity on Selected Biomarkers in Clarias gariepinus


Paraquat Toxicity on Selected Biomarkers in Clarias gariepinus

References


