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SUB-LETHAL GENOTOXICITY OF THE 1,1′-DIMETHYL-4,4′-BIPYRIDINIUM DICHLORIDE-BASED HERBICIDE (*EXPRESS*) IN JUVENILE *Clarias gariepinus*: ORGAN-SPECIFIC DNA DAMAGE IN THE LIVER, GONADS, AND GILLS

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Abstract

The increasing global demand for food, driven by rapid population growth, has intensified the application of pesticides in agriculture. While these agrochemicals enhance crop yield, their pervasive use poses profound ecological risks, particularly to aquatic ecosystems vulnerable to pesticide runoff. Express, a widely used herbicide in Nigeria, contains 1,1′-dimethyl-4,4′-bipyridinium dichloride, an active ingredient with a well-documented global footprint in weed management. This study assessed the acute and sub-lethal genotoxic effects of Express on juvenile Clarias gariepinus, focusing on the liver, gonads, and gills, using the Comet assay as a biomarker of DNA damage. Acute toxicity tests revealed a concentration-dependent increase in mortality, with Probit analysis estimating a 96-hour LC_{50} of 0.095 mL/L, underscoring the compound's high lethality. Sub-lethal exposures (0.03, 0.07, 0.10, and 0.13 mL/L) elicited marked behavioural and physiological disruptions, including hyperactivity, immobility, and skin depigmentation, with severity escalating at higher concentrations. Comet assay results demonstrated significant ($p < 0.01$) DNA strand breaks in all examined tissues, with damage patterns indicating organ-specific susceptibility. The pronounced genotoxic potential of Express raises critical concerns for aquatic biodiversity, trophic stability, and food security, as well as potential implications for human health via the aquatic food chain. These findings underscore the urgent need for stringent environmental regulations, robust ecotoxicological monitoring, and the promotion of sustainable weed management strategies to protect aquatic life and maintain ecosystem integrity.

Keywords: *1,1′-Dimethyl-4,4′-Bipyridinium Dichloride (Express), Comet assay, DNA damage, Pesticides, Agrochemicals, Genotoxicity*

Introduction

The steady growth in the world's population has driven an increase in the global demand for food and related resources. This rising demand has, in turn, contributed to a significant increase in the use of pesticides worldwide (Islam *et al*., 2022). The term pesticide refers to a diverse group of substances designed to combat pests such as weeds, insects, rats, nematodes, and mites (Rad *et al*., 2022). Pesticides occur in various forms and are further classified according to the target organism and intended use: herbicides, fungicides, and insecticides.

To meet the food needs of the world's increasing population, which stood at approximately 6.8 billion in 2009 and reached 7 billion in 2012 (Shefali *et al*., 2021), farmers have intensified their use of agrochemicals, including herbicides, fungicides, nematicides, and fertilizers (Shefali *et al*., 2021). With the global population projected to reach 10 billion by 2050, the need to increase food production to meet rising demand has become a paramount concern for many nations (Shefali *et al*., 2021).

While pesticides are essential components of modern agricultural practice, their predominantly synthetic nature raises serious concerns regarding potential risks to both human health and the environment (Islam *et al*., 2022; Rad *et al*., 2022). In developing countries, pesticides are widely adopted as a cost-effective means to increase agricultural yields, thereby enhancing food security and offering crop insurance to farmers (Sarkar *et al*., 2021). In regions with limited resources, pesticides are considered the most effective tools for protecting crops against pests and weeds that threaten livelihoods (Sarkar *et al*., 2021). However, their widespread use often results in pollution of water bodies through physical processes such as runoff, thus exacerbating environmental risks.

As highlighted by Shefali *et al.* (2021), anthropogenic activities, including agricultural, industrial, and domestic practices, are primary contributors to freshwater pollution in rivers and streams. Although pesticides are intended to control specific target organisms, extensive research shows that pesticides, industrial chemicals, heavy metals, and other toxicants also affect non-target species, with adverse effects on both humans and aquatic organisms (Shefali *et al*., 2021). Chemicals enter aquatic ecosystems through multiple pathways: runoff, atmospheric vaporization, groundwater infiltration, adsorption, and plant uptake (Jabali *et al*., 2020). Once present in aquatic habitats, pesticides exert harmful effects on fish, zooplankton, and even birds, with especially severe consequences in agricultural and urban areas.

Although manufacturers impose limits on herbicide applications, field studies consistently report concentrations in aquatic environments that exceed permissible levels (Ayanda *et al*., 2021; Oloruntoba *et al*., 2024). Fish, owing to their sensitivity to xenobiotics, are invaluable bioindicators of environmental toxicity and play a critical role in assessing ecological risks (Ayanda *et al*., 2021; Iyiola *et al*., 2024). Given their ecological and economic significance, the need to understand the impacts of pesticides on fish species has become increasingly important.

The organism of focus in this study is the African catfish (*Clarias gariepinus*), a species valued both commercially and ecologically. Known for its resilience and adaptability across diverse freshwater

systems, *C. gariepinus* serves as an excellent model organism for toxicological investigations (Ng, 2021). Three key organs were examined: the gills, gonads, and liver. The gills perform respiration, osmoregulation, excretion of nitrogenous wastes, pH regulation, and hormone synthesis (Foyle *et al*., 2020; Zimmer, 2024). Gonads are responsible for the production of sex cells (sperm in testes and eggs in ovaries), with their development influenced by hormones, temperature, sunlight exposure, and other environmental stimuli (Hsu and Chung, 2021; Brainkat, 2023). The liver, the largest and most important fish organ, is involved in nutrient assimilation, bile production, detoxification, and maintenance of metabolic homeostasis, including processing of carbohydrates, proteins, lipids, and vitamins (Moraes and Almeida, 2020). Because these organs are central to vital physiological processes, they are also the most susceptible indicators of genotoxic effects from herbicides such as Express.

'Express' herbicide is water-soluble and commonly used by Nigerian farmers to control weeds in agricultural and non-agricultural fields. Its active ingredient is 1,1′-dimethyl-4,4′-bipyridinium dichloride, commonly known as paraquat (a highly toxic quaternary ammonium compound) (Andrew *et al*., 2025). Paraquat is characterized by rapid, non-selective action: it disrupts photosynthesis, ruptures cell membranes, and desiccates foliage (Manju *et al*., 2022). This efficiency has made it a popular weed-control agent in food production (Nafi'u *et al*., 2021; Aribisala *et al*., 2022). However, paraquat runoff into aquatic systems poses a significant threat to aquatic organisms and ecosystems.

Concerns regarding the toxic effects of paraquat and similar herbicides on non-target organisms (particularly in aquatic habitats) continue to rise. Given the economic importance of *Clarias gariepinus* in Nigeria, any adverse effects on its survival could harm food security and disrupt local livelihoods. Moreover, direct chemical analyses of water and sediment alone are insufficient to evaluate the toxicity of complex chemical mixtures in living systems (Ayanda *et al*., 2021). Therefore, this study aims to bridge the knowledge gap concerning the genotoxic impacts of very low concentrations of paraquat-based herbicides on the vital organs of *C. gariepinus*.

By conducting both acute toxicity tests and comet assay experiments, this study provides new insights into the immediate and genotoxic impacts of Express on *C. gariepinus*. Beyond national implications, these findings also contribute to global discussions on herbicide safety, particularly in regions where paraquat-based products remain widely used.

Materials and Methods

Experimental Design

The species selected for this study was the African catfish (*Clarias gariepinus*). One hundred (100) juvenile catfish were obtained from a fishpond at the Faculty of Agriculture, University of Benin, Benin City. To minimize stress, the fish were transported to the laboratory in aerated containers.

Acute Toxicity Test

The fish were exposed to sublethal concentrations of the herbicide Express. Four (4) concentrations were prepared: 0.03, 0.07, 0.10 and 0.13 mL/L. Fish were considered dead when no movement was observed, and the body was either floating

horizontally at the surface or sinking to the bottom of the test medium.

To enhance the validity of results and account for variability, each concentration was tested in two independent duplicates, labelled A and B (for example, 0.1A and 0.1B). In total, eight experimental tanks were used, each containing ten fish. For control, ten juveniles were maintained in a separate tank filled only with distilled water. This served as the control group for comparison of DNA integrity between exposed and unexposed fish. The experimental solutions were renewed every 24 hours to prevent contamination by metabolic wastes. Observations were recorded throughout the study for statistical analysis.

Comet Assay

The comet assay was conducted following the alkaline single-cell gel electrophoresis protocol described by Singh *et al.* (1998). After the acute toxicity test, the gills, gonads, and liver were excised from both exposed and control fish. Each organ sample was placed in labelled Eppendorf tubes and stored in Eppendorf storage boxes to ensure proper identification and prevent mix-ups.

The harvested organs were digested with trypsin to release cellular contents and make DNA material available for analysis. Microscope slides were labelled with diamond-tipped pens for accurate sample identification. A lysing solution (containing sodium chloride, disodium EDTA, dimethyl sulfoxide (DMSO), Triton X-100, and deionized water) was prepared to disrupt cell membranes and expose DNA strands for electrophoresis.

Slides were prepared with a foundation layer of normal agarose, followed by embedding of digested cells in 0.5% low-melting agarose. A coverslip was placed to ensure uniform thickness. After this layer solidified, a final 1% agarose layer was added to protect the embedded cells from contamination. The slides were then treated with the lysing solution for 24 hours to facilitate nucleoid formation.

The electrophoresis buffer was prepared by mixing 30 mL NaOH solution and 5 mL EDTA solution, and then diluting with deionized water to a final volume of 1 L. The buffer-maintained pH conditions during electrophoresis. Electrophoresis was performed in a dark room to minimize background light, which could interfere with comet visualization. DNA fragments migrated away from nucleoids, forming a "tail" indicative of strand breaks.

After electrophoresis, Tris buffer (pH 7.4) was applied to neutralize the slides. To preserve DNA integrity, slides were fixed in cold ethanol for five minutes and stored in a freezer. Giemsa stain was applied to enhance the visualization of comet structures under a compound light microscope. The extent of DNA damage was quantified by measuring the degree of DNA migration. Images were captured using ImageJ software, with at least 50 comets analyzed per slide to ensure reliable quantification (minimum of 100 comets per slide).

Data Analysis

The data generated were analyzed using GraphPad Prism 8 and Microsoft Excel. Means and standard deviations were calculated for each group. Two-way analysis of variance (ANOVA) was employed to compare control and treatment groups, while Tukey's multiple comparison test was applied to assess differences among means. Results are presented as mean ± standard deviation, with significance set at $p < 0.01$. The

primary comet assay parameter analyzed was the olive moment.

Results

Acute Toxicity Tests

During the 96-hour exposure period, mortality rates and behavioral responses of *Clarias gariepinus* were carefully monitored across the different herbicide concentrations. The median lethal concentration (LC_{50}) was calculated using Quest Graph™ software, and probit analysis was conducted with Microsoft Excel.

For replicate group A, the LC_{50} was 0.09 mL/L; for replicate group B, the LC_{50} was 0.10 mL/L. The average LC_{50} value across replicates was therefore 0.095 mL/L. Probit values for replicate group A ranged from 4.16 at the lowest concentration to 5.84 at the highest concentration, while the LC_{50} remained 0.09 mL/L.

Table 1: Mortality response of exposed *Clarias gariepinus* (Group A)

Conc. (mL/L)	Log of Concentration	Mortality					Number of mortalities	Mortality rate	Probit value
		12 hours	24 hours	48 hours	72 hours	96 hours			
Control	-	0	0	0	0	0	0/10	0%	-
0.03	-1.52	0	0	1	1	0	2/10	20%	4.16
0.07	-1.15	0	1	1	1	0	3/10	30%	4.48
0.10	-1	0	1	1	2	2	6/10	60%	5.25
0.13	-0.89	0	1	1	2	4	8/10	80%	5.84

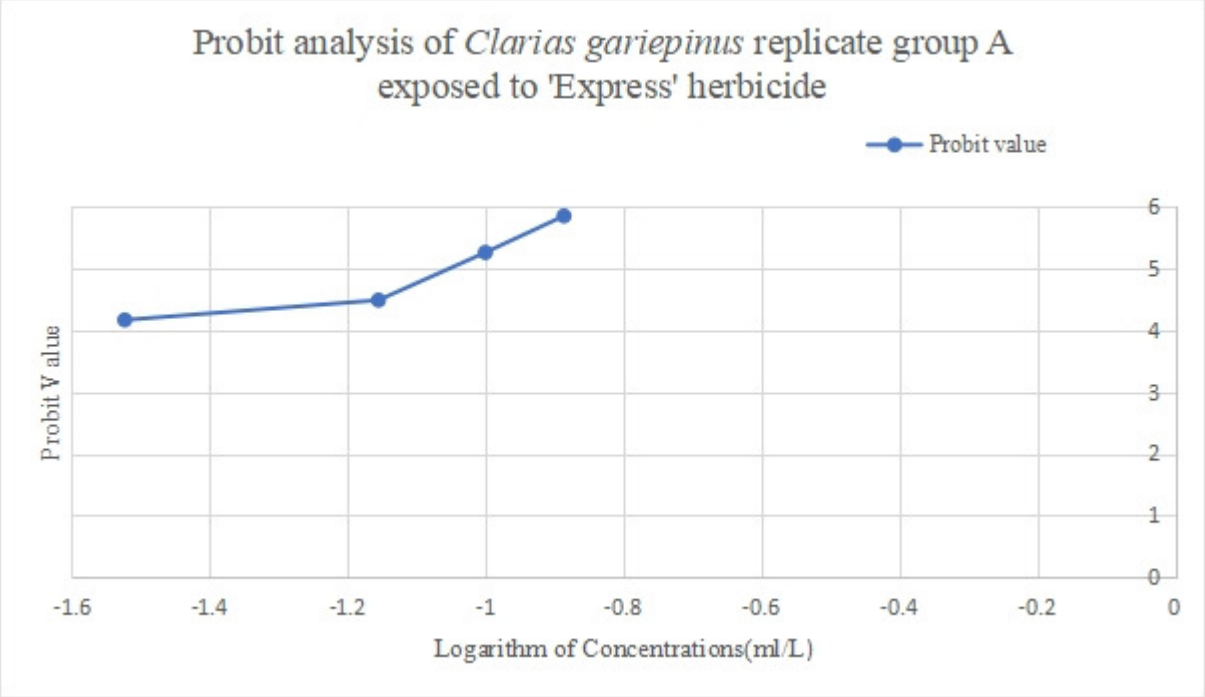

Fig. 1: Probit analysis for replicate group A

For replicate group B, probit values ranged from 4.48 at the lowest concentration to 5.84 at the highest concentration. The median lethal concentration (LC_{50}) for this group was determined to be 0.10 mL/L.

Table 2: Mortality response of exposed *Clarias gariepinus* (Group B)

Conc. (ml/L)	Log of concentration	Mortality					Number of mortalities	Mortality rate	Probit value
		12 hours	24 hours	48 hours	72 hours	96 hours			
Control	-	0	0	0	0	0	0/10	0%	-
0.03	-1.52	0	1	0	1	1	3/10	30%	4.48
0.07	-1.15	0	0	0	2	1	3/10	30%	4.48
0.10	-1	0	0	1	2	2	5/10	50%	5.00
0.13	-0.89	0	1	2	2	3	8/10	80%	5.84

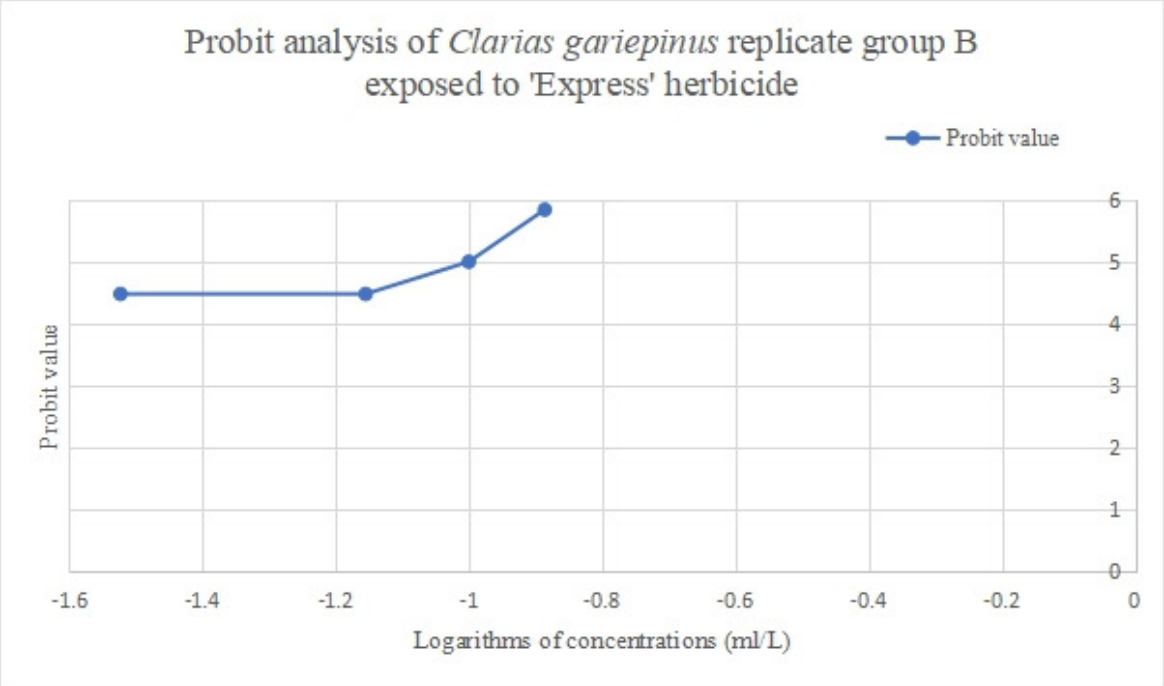

Fig. 2: Probit analysis for replicate group B

Observed Changes in Behaviour

Aside from mortality, other significant behavioural and physiological changes were observed in the test organisms. These changes were more pronounced in fish exposed to higher concentrations of Express. The most notable alterations included hyperactivity, depigmentation of skin colour (from black to pale grey), and immobility, particularly when the fish were close to death. These responses corresponded with the onset of toxic effects and were consistently more evident at higher exposure levels.

Genotoxic Assessment Using the Comet Assay

Slides were examined under a compound light microscope at ×160 magnification, and micrographs were quantified using Image J version 1.3.1.

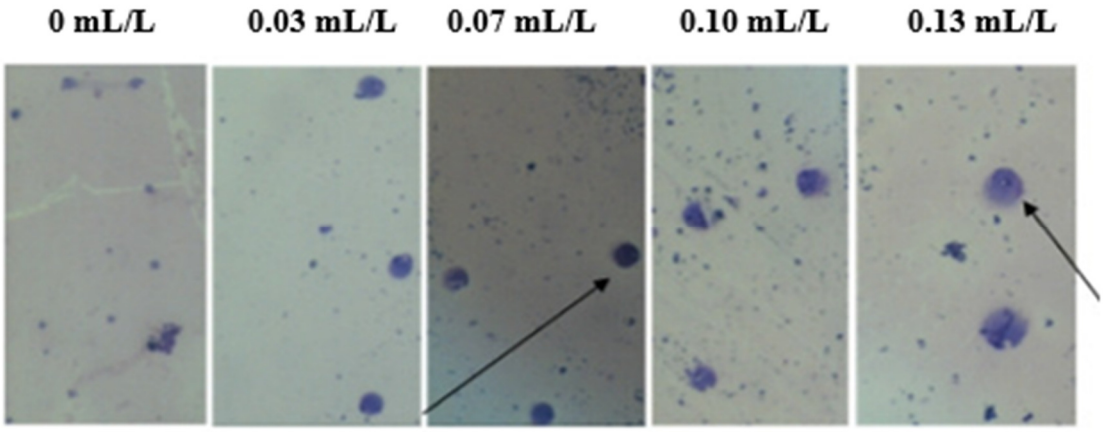

Fig. 3: Micrographs of DNA damage to gills
Micrographs of DNA damage done to the gills of *Clarias gariepinus* at concentrations 0 mL/L(control), 0.03 mL/L, 0.07 mL/L, 0.1 mL/L and 0.13 mL/L. Arrows show comets formed in gills of Clarias gariepinus treated with "Express".

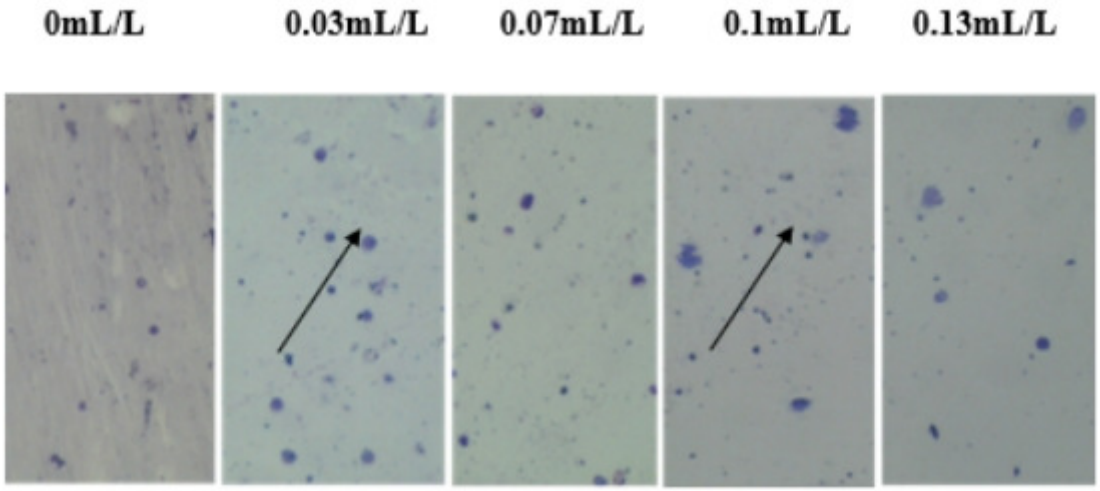

Fig. 4: Micrographs of DNA damage to gonads.
Micrographs of DNA damage done to the gonads of Clarias gariepinus at concentrations 0 mL(control), 0.03mL/L, 0.07mL/L, 0.1mL/L and 0.13mL/L**.** Arrows show comets formed in gonads of Clarias gariepinus treated with "Express".

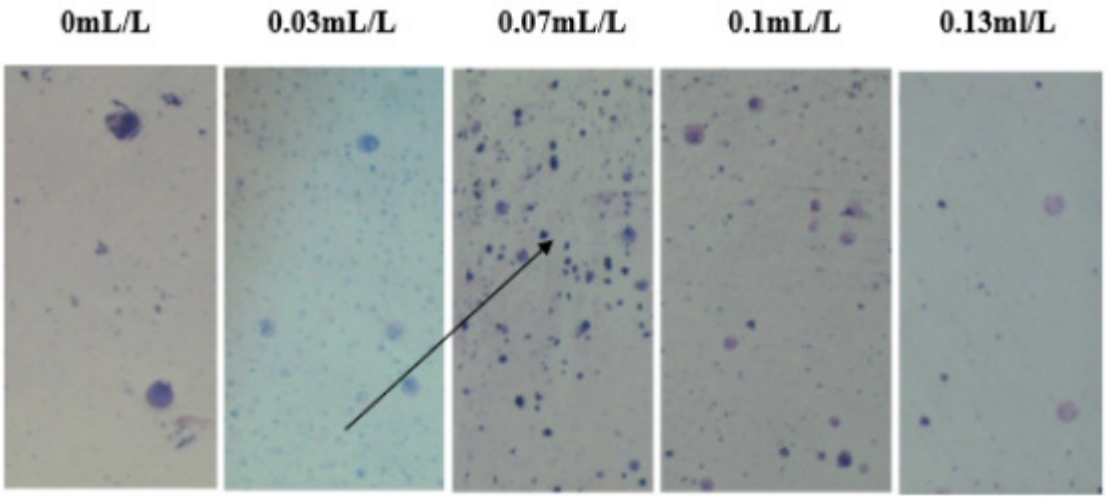

Fig. 5: Micrographs of DNA damage to the liver
Micrographs of DNA damage done to the liver of Clarias gariepinus at concentrations 0 ml/L(control), 0.03mL/L, 0.07mL/L, 0.1mL/L and 0.13mL/L**.** Arrow shows comets formed in liver of Clarias gariepinus treated with "Express".

DNA Damage in Organs of Clarias gariepinus (Graphical Representation)

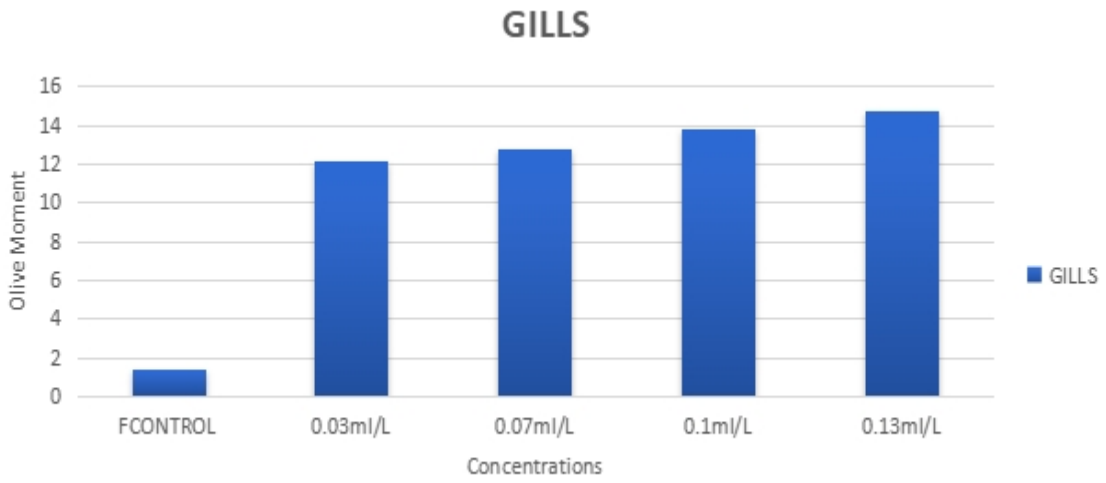

Fig. 6: Effects of 'Express' on the gills of *Clarias gariepinus*
Each bar represents the mean ± SEM (n=4) presented as the olive moment of each organ at the various concentrations
SEM – Standard error of mean

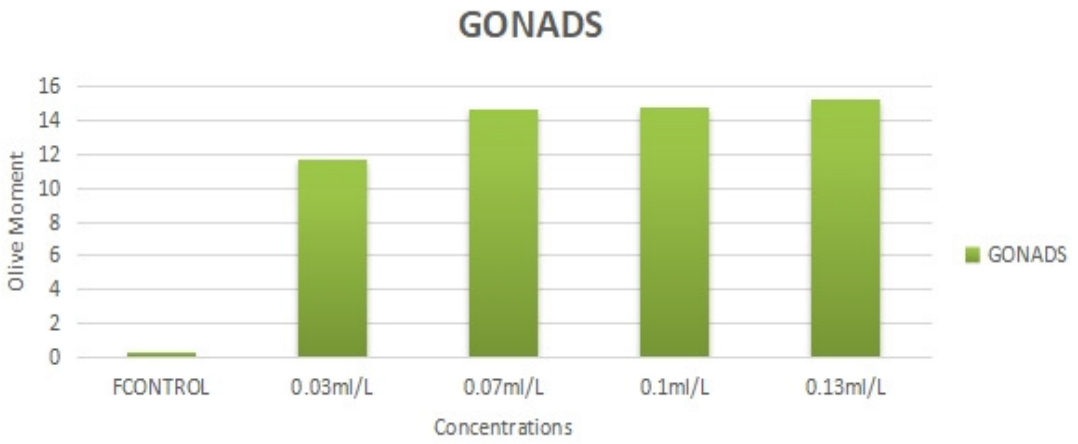

Fig. 7: Effects of 'Express' on the gonads of *Clarias gariepinus*
Each bar represents the mean ± SEM (n=4) presented as the olive moment of each organ at the various concentrations
SEM – Standard error of mean

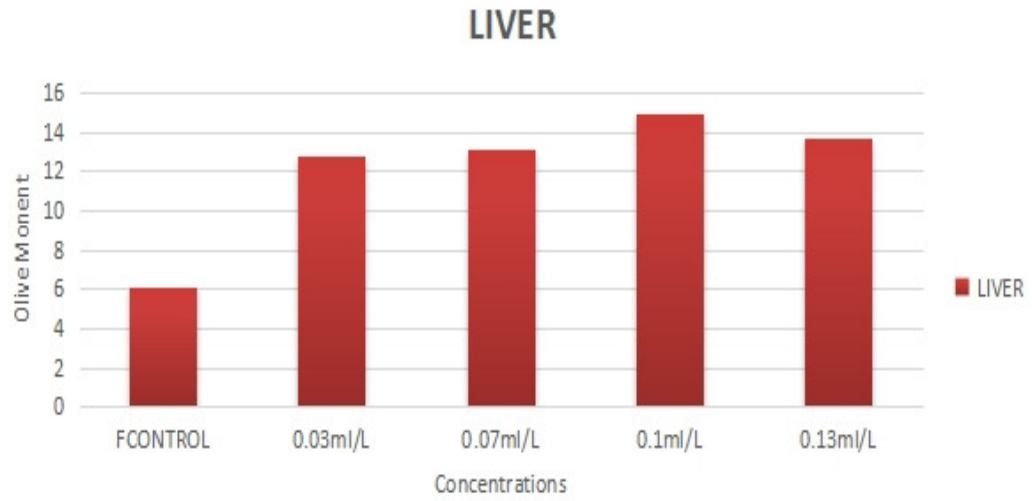

Fig. 8: Effects of 'Express' on the liver of *Clarias gariepinus*
Each bar represents the mean ± SEM (n=4) presented as the olive moment of each organ at the various concentrations
SEM – Standard error of mean

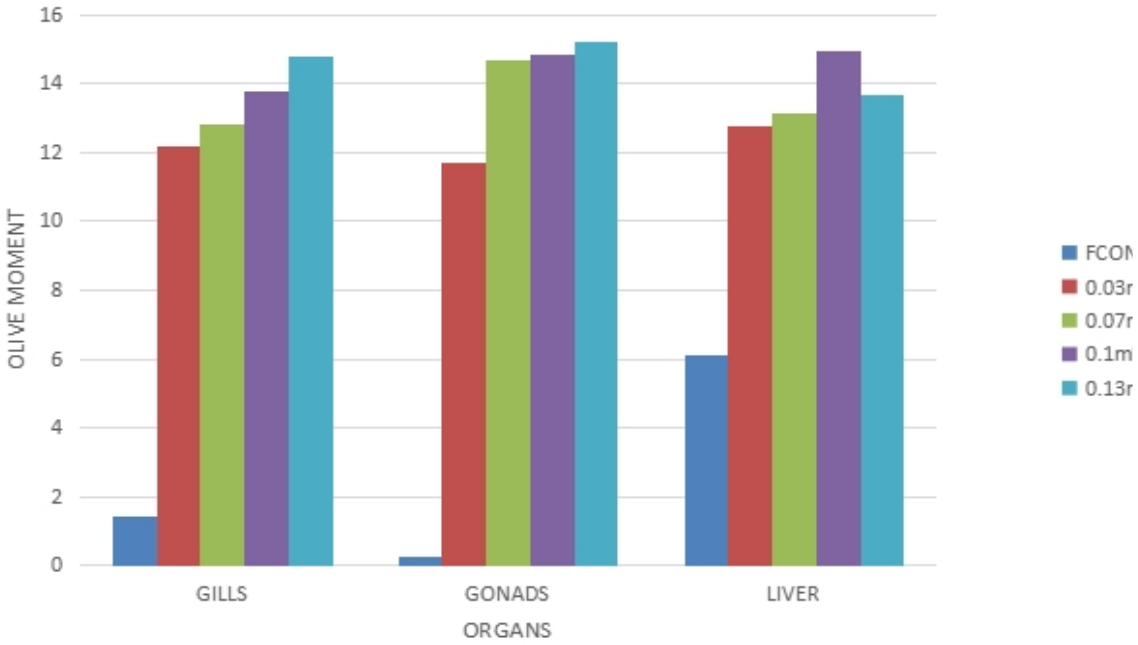

Fig. 9: Comparism of the effects of 'Express' on the gills, gonads and liver of *Clarias gariepinus*

Each bar represents the mean ± SEM (n=4) presented as the olive moment of each organ at the various concentrations, SEM – Standard error of mean

Discussion

The primary objective of this study was to assess the genotoxic effects of exposure to very low concentrations of the 1,1′-dimethyl-4,4′-bipyridinium dichloride-based herbicide, Express, on the liver, gonads, and gills of juvenile *Clarias gariepinus*. Findings from the experimental analyses offered valuable insights into the potential ecological risks associated with Express exposure in aquatic organisms and ecosystems. Results from the acute toxicity tests (Tables 1 and 2) demonstrated clear patterns of concentration-dependent effects. We observed a clear dose-dependent increase in mortality, with higher death rates recorded among fish exposed to greater concentrations of Express. This finding aligns with earlier acute toxicity studies (Ayanda *et al*., 2015; Oladokun *et al*., 2020). Probit analysis (Figures 1 and 2) confirmed the substantial lethal impact of Express on *Clarias gariepinus*, with probit values ranging from 4.16 to 5.84. The combined LC_{50} value for this study was calculated as 0.095 mL/L. These results are consistent with Ayanda *et al.* (2021), who reported a 96-hour LC_{50} of 0.07 mg/L for paraquat exposure in *Clarias gariepinus*. The similarity in LC_{50} values emphasizes the significant threat posed by Express and related herbicides to aquatic organisms. From these findings, it can be inferred that prolonged exposure to elevated concentrations of paraquat-based herbicides increases the risk of fish mortality.

Beyond mortality, other notable physiological and behavioural alterations were observed, particularly in fish

exposed to higher concentrations of Express. The most prominent changes included initial hyperactivity, followed by immobility and delayed response to external stimuli, especially as death approached. Additionally, there was a marked depigmentation of skin colour, transitioning from dark black to pale grey. These behavioural responses corroborate observations from studies reporting similar toxic effects of paraquat dichloride on freshwater fish (Ayanda *et al*., 2015; Badroo *et al*., 2020; Oladokun *et al*., 2020). The consistency of these changes across multiple studies reinforces the conclusion that behavioural alterations are reliable early indicators of toxicant stress in fish.

The comet assay results further revealed substantial DNA damage in the gills, gonads, and liver of exposed fish (Figures 3 - 9). Compared with control samples, fish exposed to Express displayed pronounced genetic damage in all three organs. These genotoxic effects are consistent with findings of concentration-dependent DNA damage in *C. gariepinus* exposed to paraquat (Ayanda *et al.,* 2021). Interestingly, in some instances, DNA damage did not increase strictly in line with concentration; for example, liver samples from fish exposed to 0.13 mL/L displayed slightly less damage than those exposed to 0.10 mL/L. This indicates complex, organ-specific responses to Express exposure. Nonetheless, both gills and gonads showed a steady upward trend in DNA damage, although not always statistically significant.

Statistical analysis revealed significant differences between the control and exposed groups, confirming a dose-dependent increase in DNA damage across the studied organs. Of particular concern is the high susceptibility of the gills, as genotoxic injury can impair respiration and osmoregulation, ultimately threatening survival (Herrero *et al*., 2018; Aruna *et al*., 2021). Similarly, gonadal damage compromises reproductive capacity, raising the risk of reduced fertility and long-term population decline in *C. gariepinus* (Hsu and Chung, 2021; Brainkat, 2023). DNA damage in liver tissue further highlights the herbicide's potential to impair vital metabolic and detoxification processes, jeopardizing overall health and resilience (Moraes and Almeida, 2020). Collectively, these findings demonstrate that even sub-lethal exposure to Express can disrupt critical biological systems, threatening not only individual fish health but also species persistence.

The ecological implications are far-reaching. As *C. gariepinus* is a key species within aquatic food webs, damage to its health and reproductive success can trigger cascading effects throughout ecosystems. Furthermore, the genotoxic potential of Express poses risks to non-target species, reducing biodiversity and disrupting ecological balance (Kadiru, *et al*., 2022). Such disruptions may manifest as habitat alteration, water quality deterioration, eutrophication, and harmful algal blooms, ultimately destabilizing aquatic environments (Bhat, 2013; Bhat *et al*., 2022; Brühl, *and* Zaller, 2019).

The consequences also extend beyond ecological boundaries to human health, economic stability, and cultural values. Herbicide residues in water sources can expose human populations to serious health risks, including cancers and reproductive disorders (Hassaan, *and* Nemr; Singh, 2021). Economically, fisheries-dependent communities may face declining fish stocks, leading to

reduced income and food insecurity (Tang *et al*., 2021). Additionally, the erosion of traditional fishing practices and cultural reliance on fish threatens community identity and heritage (Kim *et al*., 2017). Ethically, indiscriminate herbicide use raises pressing questions about environmental responsibility and intergenerational equity (Tudi *et al*., 2021).

Conclusion

This study provides clear evidence that exposure to Express herbicide (even at very low concentrations) induces significant genotoxic effects in the gills, gonads, and liver of juvenile *Clarias gariepinus*. Acute toxicity testing revealed immediate and concentration-dependent mortality, while comet assay analyses demonstrated dose-dependent DNA damage in key organs. Conclusively, these findings establish that Express poses substantial risks to the survival, reproduction, and genetic integrity of *C. gariepinus*, with wider implications for aquatic biodiversity, ecosystem stability, human health, and local economies. Preventive and regulatory strategies must therefore be prioritised to minimise the discharge of such herbicides into aquatic systems and safeguard environmental and public health.

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