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Neuro-ocular histopathology and integrated tissue injury index in Mugil cephalus following acute paraquat exposure

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Article

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Abstract

Background

Paraquat is a neurotoxic herbicide that can cross the blood-brain and blood-retinal barriers, inducing oxidative stress and cell damage. However, histopathological studies on the brain and eye of marine fish, particularly the grey mullet (*Mugil cephalus*), are limited.

Objective

This study evaluated histopathological alterations in the brain and eye of *M. cephalus* after acute sublethal paraquat exposure and developed an injury index across these organs.

Methods

Juvenile *M. cephalus* (22.79 ± 1.14 g) were exposed to sublethal paraquat concentrations (0.38, 0.76, and 1.14 mg/L; 25%, 50%, and 75% of the 96-h LC_{50} of 1.52 mg/L) for 96 h. Brain and eye tissues were processed for histology, stained with hematoxylin and eosin, and scored using the Bernet injury index.

Results

Control brains and eyes showed normal architecture. At 0.38 mg/L, no significant lesions were observed. At 0.76 mg/L, mild vacuolation appeared in the optic tectum and medulla. At 1.14 mg/L, extensive vacuolation and clear hole formation were present in the brain, with retinal vacuolation and clear holes near the optic nerve head. The injury index for brain and eye increased significantly at 1.14 mg/L ($p < 0.05$).

Conclusion

Acute paraquat exposure induces vacuolation and clear hole formation in the brain and eye of *M. cephalus*, likely mediated by ROS-induced oxidative stress. The injury index provides a comprehensive quantitative tool for assessing multi-organ toxicity, with brain, and eye. These findings highlight the neuro-ocular risk of paraquat in marine fish and the value of multi-organ histopathology in ecotoxicology.

1. Introduction

Paraquat is a bipyridinium herbicide known for its potent neurotoxicity in mammals and fish, mediated through oxidative stress and mitochondrial dysfunction [1]. The herbicide can cross the blood-brain barrier and accumulate in neural tissues, where it generates reactive oxygen species (ROS) that damage

neurons and glial cells [2]. In fish, paraquat exposure has been associated with vacuolation, neuronal degeneration, and impaired swimming behavior [3]. However, histopathological studies on the brain and eye of marine fish, particularly *Mugil cephalus*, are scarce.

The eye is an extension of the central nervous system and is similarly vulnerable to oxidative damage. Retinal vacuolation and optic nerve degeneration have been reported in fish exposed to pesticides [4]. Given that grey mullet rely heavily on vision for foraging and predator avoidance, ocular damage can have direct ecological consequences [5].

An injury index, such as that proposed by Bernet et al. [6], provides a quantitative summary of histopathological damage across tissues. Combining injury scores from the brain and eye allows an integrated assessment of paraquat's overall toxicity [7].

This study aimed to: (1) characterize histopathological changes in the brain and eye of *M. cephalus* after acute sublethal paraquat exposure, (2) compute an injury index incorporating examined organs, and (3) assess whether neural damage is consistent with oxidative stress-mediated mechanisms.

2. Materials and Methods

2.1 Fish collection, acclimation, and exposure

Juvenile *M. cephalus* (22.79 ± 1.14 g) were obtained, acclimated, and exposed to sublethal paraquat concentrations (0.38, 0.76, and 1.14 mg/L) for 96 h as described in previous studies [8]. Control fish were maintained in clean water. All conditions were identical.

2.2 Histology of brain and eye

After 96 h, fish were anesthetized by immersion in a clove oil solution and euthanized by ten times the anesthetic concentration (60 mg/l to 600 mg/l) [9], and the brain and eyes were dissected and fixed in 10% formalin. Tissues were processed routinely, embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin and eosin. For the brain, sections were taken at the level of the optic tectum and medulla oblongata. For the eye, sagittal sections through the optic nerve and retina were prepared. Histopathological alterations (vacuolation, clear holes, neuronal shrinkage) were scored semi-quantitatively (0 = absent, 1 = mild, 2 = moderate, 3 = severe).

2.3 Injury index

The injury index for the brain and eye was calculated using the Bernet et al. [6] method. For each lesion, a score value (0–6) and an importance factor (1–3) were assigned. The organ index (I□□□) was Σ(score × importance factor) for all lesions within that organ. The integrated (total) injury index for each fish was the sum of the individual organ indices. Mean integrated indices were compared across treatment groups.

2.4 Ethical statement

All experimental procedures were approved by the Animal Ethics Committee of Chabahar Maritime University (approval number: CMU-AEC-2022-018). Confirming that all experiments were performed in accordance with relevant guidelines and regulations.

2.5 Statistical analysis

Data were expressed as mean ± SE. One-way ANOVA followed by Tukey's post-hoc test was performed using SPSS v.26.0. Significance was set at $p < 0.05$.

3. Results

3.1 Eye histopathology

Control eyes showed intact retina with well-organized layers (ganglion cell, inner plexiform, inner nuclear, outer plexiform, outer nuclear, and photoreceptor layers). At 0.38 and 0.76 mg/L, no significant lesions were observed. At 1.14 mg/L, vacuolation appeared in the inner plexiform and ganglion cell layers, and occasional clear holes were present near the optic nerve head (Fig. 1A, B). The injury score for the eye increased significantly at 1.14 mg/L ($p < 0.05$) (Table 1).

3.2 Brain histopathology

Control brains showed normal neuronal architecture with intact nuclei and cytoplasm. At 0.38 mg/L, occasional small vacuoles were observed in the optic tectum, but overall morphology was near normal. At 0.76 mg/L, vacuolation increased in number and size, with some clear holes appearing in the medulla. At 1.14 mg/L, extensive vacuolation and clear hole formation were present in both the optic tectum and medulla, with loss of neuronal organization (Fig. 1C, D). The injury score for the brain increased significantly at 1.14 mg/L ($p < 0.05$) (Table 1).

Table 1. Injury scores (mean ± SE) for the brain and eye in *M. cephalus* exposed to paraquat.

Organ	Control	0.38 mg/L	0.76 mg/L	1.14 mg/L
Brain	1.2 ± 0.3	2.5 ± 0.5	4.1 ± 0.6*	7.8 ± 0.9*
Eye	0.8 ± 0.2	1.1 ± 0.3	2.0 ± 0.4	5.3 ± 0.7*

*Significantly different from control ($p < 0.05$)

3.3 injury index

Semi-quantitative scoring of individual histopathological alterations confirmed that vacuolation was the predominant lesion in both brain and eye (Table 2). In the brain, vacuolation increased from mild at 0.76 mg/L (1.5 ± 0.4) to severe at 1.14 mg/L (2.8 ± 0.2). Clear holes appeared only at 1.14 mg/L (2.2 ± 0.3), while neuronal shrinkage was mild even at the highest concentration (1.0 ± 0.3). In the eye, vacuolation

became significant at 1.14 mg/L (2.1 ± 0.4), while clear holes and neuronal shrinkage were observed only at the highest concentration and remained mild. These individual alteration scores together contributed to the composite injury index reported in Table 1.

Table 2. Semi-quantitative scores (mean ± SE) for individual histopathological alterations in the brain and eye of *M. cephalus* exposed to paraquat for 96 h. Scores: 0 = absent, 1 = mild, 2 = moderate, 3 = severe.

Organ	Alteration	Control	0.38 mg/L	0.76 mg/L	1.14 mg/L
Brain	Vacuolation	0.4 ± 0.2	0.8 ± 0.3	1.5 ± 0.4*	2.8 ± 0.2*
	Clear holes	0.0 ± 0.0	0.2 ± 0.1	0.6 ± 0.2	2.2 ± 0.3*
	Neuronal shrinkage	0.2 ± 0.1	0.3 ± 0.1	0.5 ± 0.2	1.0 ± 0.3*
Eye	Vacuolation	0.3 ± 0.1	0.4 ± 0.1	0.8 ± 0.2	2.1 ± 0.4*
	Clear holes	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 0.2*
	Neuronal shrinkage	0.1 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.9 ± 0.3*

*Significantly different from control ($p < 0.05$, Tukey's test)

4. Discussion

This study provides the first histopathological evidence of neuro-ocular damage in *M. cephalus* exposed to sublethal paraquat. Brain vacuolation and clear hole formation, particularly at 1.14 mg/L, indicate neuronal degeneration and loss of tissue integrity. These lesions are consistent with paraquat-induced neurotoxicity reported in other fish species [1,2]. Vacuolation likely results from mitochondrial swelling, endoplasmic reticulum stress, or fluid accumulation following cell death driven by ROS-induced oxidative stress [10]. The optic tectum, a region critical for visual processing, was notably affected, which may impair prey capture and predator avoidance.

The eye lesions, though less severe than in the brain, are significant. Vacuolation in the inner plexiform and ganglion cell layers suggests damage to the retinal circuitry [4]. The integrity of the blood-retinal barrier may be compromised by oxidative stress, allowing paraquat to reach the retina. In fish, the retina has a high metabolic rate and is rich in polyunsaturated fatty acids, making it particularly vulnerable to lipid peroxidation mediated by ROS [5]. The absence of significant lesions at lower concentrations suggests a threshold effect, with the eye being more resistant than the brain.

The injury index and semi-quantitative scoring provide a comprehensive view of paraquat's multi-organ toxicity. Damage in the brain and eye underscores the systemic distribution of paraquat. The organ-specific injury pattern aligns with the known pharmacokinetics of paraquat: it is rapidly absorbed, distributed to highly perfused organs, and retained in tissues with high oxidative capacity [7].

Mechanistically, paraquat's redox cycling generates ROS that deplete cellular antioxidants and initiate lipid peroxidation, ultimately leading to vacuolation and neuronal death [11]. Although we did not measure antioxidant levels in this study, the observed histopathology is fully consistent with oxidative stress-mediated damage. The vacuolation pattern in brain and eye is similar to that reported in other fish exposed to ROS-inducing toxicants [10].

Comparisons with other studies reinforce our findings. Hamed et al. [3] reported vacuolation in the brain of *Oreochromis niloticus* exposed to paraquat, while El-Shenawy et al. [12] observed neuronal degeneration in *Clarias gariepinus*. The present study extends these observations to a marine/euryhaline species and includes the eye, which has been rarely examined. The use of a standardized injury index facilitates cross-study comparisons.

Ecologically, neuro-ocular damage in grey mullet could have population-level effects. Impaired vision reduces foraging efficiency and increases vulnerability to predation. Neurological damage may disrupt swimming coordination and migratory behavior, critical for this species. Given that grey mullet are important for both capture fisheries and aquaculture in Iran, paraquat contamination in coastal areas could threaten local stocks.

5. Conclusion

Acute exposure to sublethal paraquat induces histopathological damage in the brain and eye of *M. cephalus*, characterized by vacuolation and clear hole formation. The injury index reveals a concentration-dependent increase in the brain and eye, showing significant damage at the highest concentration. These findings highlight the neuro-ocular risk of paraquat in marine fish and emphasize the need for monitoring neural and ocular histopathology in ecotoxicological assessments. The injury index serves as a valuable tool for quantifying multi-organ toxicity and informing environmental regulation.

Declarations

Conflict of interest

The authors declare no conflicts of interest.

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Author Contribution

all authors contributed to the design and implementation of the research, to the analysis of theresults and to the writing of the manuscript

Data Availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request

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Figures

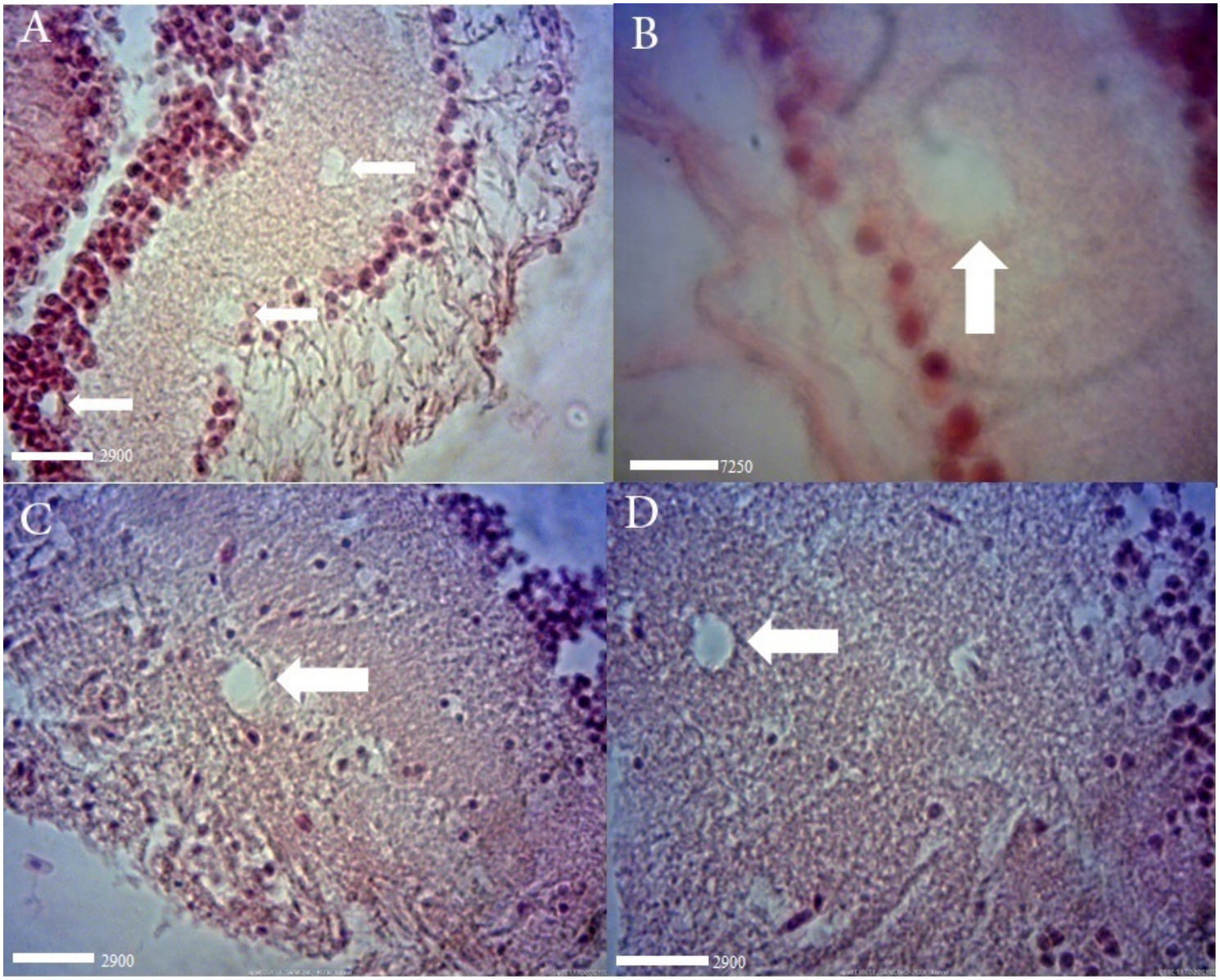


Figure 1

Fig 8. A thin section of the eye and brain of *M. cephalus* (H&E). (A, B) vacoulation in the eye exposed to 1.14 mg/L paraquat. Barin of mullet (C, D) at 75% $LC_{50/}$96hrs (1.14 mg/L).