Journal of Fisheries & Livestock Production

Hassan, et., J Fisheries Livest Prod 2015, 3:2
DOI: 10.4172/2332-2608.1000131

Research Article Open Access

Behavioral and Histopathological Changes of Common Carp (*Cyprinus carpio*) Exposed to Paraquat

Hassan M[1,2]*, Shah Norhan NA[1], Mohd Daud H[3], Chong JL[4,5], Abd Halim Shah MM[6] and Karim NU[1,2]

[1]School of Fisheries and Aquaculture Sciences, University Malaysia Terengganu, 21030, Kuala Terengganu, Terengganu, Malaysia
[2]Institute of Tropical Aquaculture, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia
[3]Department of Veterinary Clinical Studies, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[4]Kenyir Research Institute, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia
[5]School of Marine and Environmental Sciences, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia
[6]School of Fundamental Science, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia

Abstract

The common carp (*Cyprinus carpio*) was exposed to 0, 10, 20, 30, 40 and 50 mg/L of paraquat for 96 hours under laboratory conditions. The behavioral and histopathological changes of *C. carp* in response to paraquat exposure. The 96H lethal concentration (LC_{50}) value are 26.07 mg/L. Treated fish exhibited agitated, respiratory distress and abnormal nervous behavior. Hyperplasia, oedema, pyknosis and necrosis in gills, liver and kidney were observed in the histopathological studies.

Keywords: Behavioral changes; Histopathological changes; Toxicity

Introduction

The human activities such as agricultural has exploited the environment and disturbed the ecological balance and the living organisms and also human. Herbicide has been used in agriculture to maintain their crops from infestation of weeds without using high cost labor. Paraquat is a highly toxic herbicide and is highly used in Malaysia [1,2]. Since the 60's, paraquat was used in Malaysian rubber plantation and paddy field for land preparation [2,3]. The non-selective properties of paraquat can negatively affect the aquatic organisms, especially fish, which will lead to death if left untreated [4]. The behavioral study affect by paraquat is important to understand the sensitivity of the fish with the range of contaminants and the adaptation. Thus, behavior toxicology is useful indicator of sublethal contamination and the endpoints frequently occurred below the lethal concentration. Besides, the changes of the behavior can be indicator for lesions in vital organs such as gills, kidney and liver. Actually, the acute toxicity values of several pesticides products for different fish species have been reported by many researchers. Hence, this study was conducted to determine the behavioral and histopathological changes of paraquat exposure to *Cyprinus carpio*.

Materials and Methods

Juvenile *Cyprinus carpio* with a mean length of 3.7 ± 0.42 cm and weight of 0.82 ± 0.37 g were acclimatized for a week. During this period, commercial pallet was given at 5% body weight and health status was monitored. Prior to the test, the fishes were starved for 24 hours. This experimental design involved in introducing the fishes into tanks containing different concentration of paraquat to examine the behavior and histological changes of the fish organs.

A static non-renewal bioassay method was conducted throughout the lethal concentration (LC_{50}) 96H test [5]. Ten healthy fishes were exposed to paraquat per concentration (0, 10, 20, 30, 40 and 50 mg/L) with triplicate. Behavior analysis was completed by observing the visual changes of the *C. carpio* for 6 hour interval in response to paraquat. Those responses are agitated behavior, respiratory distress and abnormal nervous behavior as shown in Tables 1-3. Throughout the test, dead fish was removed immediately to prevent water contamination [5,6]. The dead fishes were dissect and observed the gross pathological changes of the vital organs.

Gills, liver and kidney were harvested and preserved with 10% buffer formalin. Samples underwent a standard tissue processing

Clinical signs	Paraquat concentration (mg/L)					
	0	10	20	30	40	50
Aggression	-	+	+	++	+++	+++
Jumping	-	-	-	-	-	-
Stunted Posture	-	+	+	++	+++	+++
FSBM	-	+	+	++	++	+++
Erratic swimming	-	+	+	++	+++	+++

Frequent Surface to Bottom Movement (FSBM), None (-), Weak (+), Moderate (++), Strong (+++)

Table 1: Agitated of *C. caprio* when subjected to paraquat for 96H LC_{50}.

Clinical signs	Paraquat concentration (mg/L)					
	0	0	20	30	40	50
Opercula movement	-	+	+	++	+++	+++
Air gulping	-	+	+	++	+++	+++
VPES	-	-	-	-	-	-
EMS	-	-	+	++	+++	+++

Ventral Posture with Extended Snout (VPES), Excessive Mucus Secretion (EMS), None (-), Weak (+), Moderate (++), Strong (+++).

Table 2: Respiratory distress of *C. caprio* when subjected to paraquat for 96H LC_{50}.

Clinical signs	Paraquat concentration (mg/L)					
	0	10	20	30	40	50
SSM	-	-	-	+	++	+++
State of motionless	-	+	+	++	+++	+++
Sudden darts	-	+	+	++	+++	+++
DP	-	-	+	+	++	+++
Death	-	+	+	++	+++	+++

Sluggish and Swirling Motion (SSM), Different Postures (DP), None (-), Weak (+), Moderate (++), Strong (+++)

Table 3: Abnormal nervous behavior of *C. carpio* when subjected to paraquat for 96H LC_{50}.

***Corresponding author:** Marina Hassan, School of Fisheries and Aquaculture Sciences, University Malaysia Terengganu, 21030, Kuala Terengganu, Terengganu, Malaysia, Tel: +609 668 3050; Fax: +609 668 4949; E-mail: marina@umt.edu.my

Received October 06, 2014; **Accepted** April 28, 2015; **Published** May 08, 2015

Citation: Hassan M, Shah Norhan NA, Shah Norhan H, Karim NU (2015) Behavioral and Histopathological Changes of Common Carp (*Cyprinus carpio*) Exposed to Paraquat. J Fisheries Livest Prod 3: 131. doi: 10.4172/2332-2608.1000131

Concentration (mg/L)	Average mortality percentage (%)
0	0
10	35
20	45
30	50
40	70
50	100

Table 4: The determination of LC_{50} value of the effects of concentration of paraquat to the *C. carpio* mortality for 96H.

procedure with hematoxylene and eosin (H&E) staining and observed via a microscope [6].

Results

Based on linear regression analysis, value of 96H LC_{50} was 26.07 mg/L with the mortality increased as paraquat concentration increased. The effects of paraquat concentration towards *Cyprinus carpio* mortality were tabulated as in Table 4. The highest mortality percentage is 100% (50 mg/L) and the lowest is 35% (35 mg/L).

Behavior varies toward different paraquat concentrations and abnormal behavior in the control group was absent (Tables 1-3). Those behavior changes are agitated, respiratory distress and abnormal nervous behavior; aggression, stunted posture, erratic behavior, opercular movement, air gulping, motionless and sluggish and swirling motion. In the 10 and 20 mg/L, minimal behavior changes occurred compared to higher concentration. Both 40 and 50 mg/L, showed the strongest behavior changes. Throughout the observation, no ventral posture extended snout (VPES) and jumping behavior recorded. Behavioral changes of *C. carpio* to paraquat were recorded as in Tables 1-3.

Histopathological lesions were present in gills, liver and kidney due to paraquat and absent in the control treatment. Epithelial lifting in the gills presence throughout 10, 40 and 50 mg/L treatment. The fused secondary lamella and hypertrophy presented only in 20 mg/L. Necrosis started to show up in 30 mg/L treatment. The aneurysm and oedema presented in 50 mg/L treatment. The liver in the control treatment shown no histopathological alterations and the cells are normal and systematically arranged. Hepatocyte with regular nucleus shape and pyknosis was presented in 10 mg/L treatment. In 20 mg/L treatment, nuclear degeneration and bile stagnation was observed. Severe necrosis and aggregation of melanomacrophages was observed at 30, 40 and 50 mg/L treatments.

No form of histopathological lesions formed in kidney of control treatment, whereas pyknosis and hydropic degeneration were observed at 10 mg/L treatment. Mild necrosis were observed throughout 20 to 30 mg/L treatments and cell hyperthropy presented in 30 mg/L treatment. Glomerulus expansion was observed at 40 mg/L treatment and irregular nucleus shape and tubular lumen inclusion presented in 50 mg/L. Based on these observations, severity of tissue alterations increased as paraquat concentration increased.

Discussion

The toxicity test was used to derive severity of behavioral analysis and histoptahological effects toward xenobiotic. The 96 h LC_{50} value of paraquat was 26.07 mg/L for *Cyprinus carpio*. Differences in LC_{50} value between species is due to the species-specific toxicity effect. Behavior observation is frequently used in a toxicity test because of the sensitive indicators of toxicant exposure [7]. In this study, no abnormal behavioral changes in control compared to the treated fishes. Agitated behavior, respiratory distress and abnormal nervous behavior exhibited in this study was observed.

The behavior changes are most sensitive indication to the toxic effects. These results showed that the mean of severity of the behavioral changes for every 6 hours until 96 hours observations. The behavioral score showed stronger with increase the concentration such as aggression, stunted posture, erratic swimming and more frequently move at the bottom. These signs indicated to the toxic irritation and the fish struggled in an attempt to get relieved from the stressful environment. Behavior such as increase opercular movement and air gulping showed that lack of oxygen. The fish used a lot of energy to adapt in the environment. Besides, the toxic was irritating the gills which are exposed directly to the environment. The irritation caused gill damages and excessive of mucous production. The fish mortality occurred due to pesticide exposure mainly depends upon its sensitivity to the toxicants and behavior of the pesticides. Osti et al. [8] observed that the behavioral changes such as swimming patterns showed that sign of neurotoxicity. The changes frequently observed earlier than mortality. In this study, 100% mortality occurred at paraquat concentration 40 to 50 mg/L. These aggressions toward the toxic environment causes lethargy and loss of equilibrium. Fish exhibited erratic swimming in order to exit the toxic environment [9] . In some cases, the fish remained stabilized, but respiratory distress increased towards the end [10]. Excessive mucus secretion in gills inhibits the gaseous exchangeability, thus hyperventilation occurred [6,9,10]. In order to compensate oxygen deficiency and direct contact towards the toxic environment, air gulping activity was observed [11].

Epithelial lifting and hyperplasia lowered the rate of gaseous exchange due to its natural mechanism to increase the pollutant-blood diffuse distance [12], along with necrosis and excessive mucus secretion, oxygen deficiency occurred. The liver is the main detoxification center of the body which is carried out by the hepatocytes [13], alteration in liver is the main indicator of toxic environment. Multiple pyknosis and necrosis of hepatocytes were observed in exposed liver, thus preventing detoxification and inability to generate new cells [14] . The lesions started to develop in the lowest dosage, which proved paraquat's severity towards *Cyrinus carpio*. Similar findings were reported in *Oreochromis niloticus* [14] , *Clarias gariepinus* [15] and *Trichogaster trichopterus* [16] where the lesions in liver started to develop lower than 2 mg/L. In the present study, pyknosis, hydropic degeneration, necrosis and tubular lumen occlusion were observed. These lesions in agreement with Cengiz [17], Mishra and Mohanty [18] and Rahman et al., [19] with the exposure of deltamethrin, chromium and diazinon. These lesions indicate a responsive action between the toxic environment and the fish, where death is an outcome due to inability to acclimatize.

Based on our findings, both behavior and histopathological are related to each other. The concentration paraquat contamination can be as indicator to the severity of lesions in the tissue vital organs such as liver, kidney and gills. The physiological changed was showed the behavioral signs as catogorised in Tables 2-4. The death showed that the vital organs collapse.

References

1. Watts M (2011) Paraquat. Pesticide Action Network Asia and The Pacific. Penang.
2. Mohd-Fuad MJ, Junaidi AB, Habibah A, Hamzah J, Toriman ME, et al. (2012) The Impact of Pesticides on Paddy Farmers and Ecosystem. Advances in Natural and Applied Sciences 6: 65-70.
3. Isenring R, Neumeister L (2011) Paraquat: Unacceptable health risks for users. (3rd edn), Berne Declaration, Pesticide Action Network UK, PAN Asia and The Pacific.

4. Eisler R (1990) Paraquat hazards to fish, wildlife and invertebrates: A synoptic review. Contaminant Hazard Reviews Report 22, Biological Report 85.

5. Anur AN, Safiah J, Ikhwanuddin M, Marina H (2011) Effects of a methanolic extract of *Melaleuca cajuputi* on African catfish (*Clarias gariepinus*). Proceedings of International Fisheries Symposium, 3rd-5th October, Kuala Terengganu.

6. Hassan M, Zakariah M, Wahab W, Muhammad S, Idris N (2013) Histopathological and Behavioral Changes in Oreochromis sp, after Exposure to Different Salinities. J Fisheries Livest Prod.

7. Sloman K, McNeil P (2012) Using physiology and behaviour to understand the responses of fish early life stages to toxicants. J fish biol 81: 2175-2198.

8. Osti SC, Varoli FMF, Matushima ER, Bernardi MM (2007) Comparative studies of delthametrin acute toxicity in exotic and Brazilian fish. J Braz Soc Ecotoxico 2: 101-106.

9. Benli AÇK, Özkul A (2010) Acute toxicity and histopathological effects of sublethal fenitrothion on Nile tilapia, *Oreochromis niloticus*. Pesticide biochemistry and physiology 97: 32-35.

10. Babatunde MM, Oladimeji AA, Balogun JK (2001) Acute toxicity of gramoxone to *Oreochromis niloticus* (Trewavas) in Nigeria. Water, Air, and Soil Pollution 131: 1-10.

11. Patil V K, David M (2008) Behaviour and respiratory dysfunction as an index of malathion toxicity in the freshwater fish, *Labeo rohita* (Hamilton). Turk J Fish Aquat Sci 8: 233-237.

12. Karan V, Vitorović S, Tutundžić V, Poleksić V (1998) Functional enzymes activity and gill histology of carp after copper sulfate exposure and recovery. Ecotoxicol Environ Saf 40: 49-55.

13. Gartner LP, Hiatt JL (2013) Color Atlas and Text of Histology. Lippincott Williams and Wilkins.

14. Ayoola SO (2008) Toxicity of glyphosate herbicide on Nile tilapia (Oreochromis niloticus) juvenile. African Journal of Agricultural Research 3: 825-834.

15. Ayoola S, Ajani EK (2008) Histopathological effects of Cypermethrin on Juvenile African catfish (Clarias gariepinus). World Journal of Biological Research 1: 362-367.

16. Banaee M, Davoodi M, Zoheiri F (2013) Histopathological changes induced by paraquat on some tissues of gourami fish (*Trichogaster trichopterus*). Open Veterinary Journal 3: 36-42.

17. Cengiz EI (2006) Gill and kidney histopathology in the freshwater fish *Cyprinus carpio* after acute exposure to deltamethrin. Environmental Toxicology and Pharmacology 22: 200-204.

18. Mishra AK, Mohanty B (2008) Acute toxicity impacts of hexavalent chromium on behavior and histopathology of gill, kidney and liver of the freshwater fish, *Channa punctatus* (Bloch). Environmental Toxicology and Pharmacology 26: 136-141.

19. Rahman M, Hossain Z, Mollah M, Ahmed G (2002) Effect of Diazinon 60 EC on *Anabas testudineus*, *Channa punctatus* and *Barbodes gonionotus*. Naga 25: 8-12.