Original Article

Effects of Sub-Lethal Toxicity of Paraquat on Blood Biochemical Parameters of Common Carp, *Cyprinus carpio* (Linnaeus, 1758)

Mahdi Banaee, Behzad Nemadoost Haghi, Somayeh Tahery, Shima Shahafve, Maryam Vaziriyan*

Received: 04.04.2016 *Accepted: 04.05.2016*

ABSTRACT

Background: Paraquat is a non-selective contact herbicide, widely used to control weeds in agriculture farms and aquatic plants in surface waters. Paraquat can have adverse effects on the health of aquatic organisms. The aim of this study was to detect the alterations in the blood biochemical parameters of common carp exposed to sub-lethal concentrations of commercial formulations of paraquat.

Methods: The fish were exposed to sub-lethal concentrations (0.2 and 0.4 $mg.L^{-1}$) of paraquat for 21 days. Biochemical parameters including glucose, total protein, albumin, globulin, creatinine, triglyceride and cholesterol levels, and aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), creatine phosphokinase (CK), alkaline phosphatase (ALP) and γ-glutamyl transferase (GGT) activities were measured.

Results: A significantly increase was observed in AST activity in fish exposed to 0.4 $mg.L^{-1}$ paraquat. Paraquat caused a significant ($P < 0.05$) increase in plasma ALT, LDH and CPK activities and plasma creatinine levels. Although ALP activity significantly ($P < 0.05$) increased in fish exposed to 0.2 $mg.L^{-1}$ paraquat, the fish exposed to 0.4$mg.L^{-1}$ paraquat exhibited a significant ($P < 0.05$) decrease in ALP activity. A significant ($P < 0.05$) decrease in GGT activity, total protein, albumin and globulin levels as well as cholesterol and triglyceride levels was observed in fish exposed to sub-lethal concentrations of paraquat.

Conclusion: Exposure to sub-lethal concentrations of paraquat may cause changes in blood biochemical parameters in common carp.

Keywords: Biochemical parameters, Common carp, Paraquat.

IJT 2016 (6): 1-5

INTRODUCTION

A major concern with any agrochemical compound such as herbicides is its potential to leach from the soil into surface or groundwater ecosystems that are used as drinking water sources. Paraquat (1, 1'-dimethyl, 4, 4'-bipyridinium dichloride) is used to control weeds in agriculture farms and aquatic ecosystems. Therefore, application of paraquat in water ecosystems may have negative impacts on the health of aquatic organisms [1-4].

Paraquat residues in surface water may be absorbed through the gills, skin and digestive system [1]. Absorbed paraquat is distributed via the blood to all organs and tissues of the fish. Due to the lipophilic property of this herbicide, it accumulates mainly in fatty tissues. Although the toxicity of paraquat for fish is low, exposure to high doses or prolonged contact with low concentrations of paraquat may also cause a severe toxicity to vital organs, primarily the liver, gills and kidney [1-4]. Toxicological properties of paraquat are attributed to its ability to produce reactive oxygen species such as superoxide anion that may directly or indirectly cause cell death [2, 5]. Damage to cells or cell death may lead to changes in blood biochemical parameters [6]. Therefore, the main hypothesis for this study was that exposure to sub-lethal concentrations of paraquat has significant effects on common carp, *Cyprinus carpio*.

The study of blood biochemical parameters could be a useful tool to diagnose toxic effects in target organs to determine the physiological status of fish exposed to herbicides [7].

The aim of this study was to identify changes in the blood biochemical parameters of common carp (*C. carpio*) exposed to sub-lethal concentrations of commercial formulations of paraquat.

Department of Aquaculture, Behbahan Khatam Al-anbia University of Technology, Khuzestan Province, Iran.
*Corresponding Author: E-mail: mahdibanaee2@gmail.com

MATERIALS AND METHODS

Chemical Materials

Paraquat (Gramoxone), 20%, was obtained as a commercial preparation (Jiangsu Hai Bang Pharmaceutical Co., China, imported by Iran). All biochemical kits were purchased from Pars Azmun Co, Iran.

Experimental Animals

Healthy common carp (*C. carpio*) with the mean weight of 45 ± 5 g (mean ± SD) were used according to the National Ethical Framework for Animal Research in Iran [8]. The fish were purchased from a local fish farm and acclimated to laboratory conditions for 2 weeks before the experiments. Fish were randomly divided into 9 plastic tanks (80 liter) supplied with oxygenated water maintaining constant dissolved oxygen at 6.5 ± 0.5 mg/L, temperature at 24 ± 2 °C, pH at 7.4 ± 0.2, and natural photoperiod. During the acclimation and all experimental tests, fish were fed commercial carp pellets (Beyza Fedd Mill Co. Iran) at the manufacturer's recommended rate.

Sub-Lethal Toxicity Experiments

One hundred eight common carp were randomly distributed in nine 80-L tanks (3 treatments with three replicates) and the experiment was run for 21 day sub-lethal toxicity tests. Every tank had 12 fish exposed to solutions with the following concentrations of paraquat: 0.0 (control), 0.2 mg and 0.4 mg per liter. Sub-lethal concentrations were selected according to the lethal concentration of paraquat required to kill 50% of the common carp [9]. Tanks were cleaned by siphoning and 40% of the water was exchanged daily to reduce the build-up of metabolic wastes and the herbicide was again added to maintain paraquat concentrations constant (equivalent to 0.2 and 0.4 mg per liter).

Sampling and Analysis of Blood Biochemical Parameters

The fish were starved for 24 h before sampling. After the 21-day exposure period, 12 fish from each treatment (4 fish from each tank) were taken out for sub-lethal toxicity studies. Each group of fish was harvested using a scoop net and placed into an anesthetic solution (200 $mg.L^{-1}$ clove powder). Blood sample was collected from the caudal vein using heparinized syringes, centrifuged at 6000 × g for 10 min and stored at -25 °C.

All blood biochemical parameters were determined using a UV-visible spectrophotometer (UNICO 2100) and standard biochemical reagents (Pars Azmun Company, Tehran, Iran). Each blood biochemical parameter was measured by a certain method. Total plasma protein was measured at 540 nm by the Biuret reaction. Albumin determination was based on the dye-binding properties of plasma albumin with bromocresol green. An increase in the blue-green color was measured at 630 nm. The plasma globulin was based on the ratio of albumin to total protein [10]. Plasma glucose was measured by the glucose-oxidase method at 500 nm [11]. Plasma cholesterol levels were determined by the CHOD-PAP method at 510 nm, triglyceride levels by GPO-PAP method at 546 nm [12] and creatinine by the JAFFE method and at 510 nm [13]. The activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in plasma was determined by NADPH consumption and its conversion to NAD^+ at 340 nm. Gamma-glutamyl transferase (GGT) activity is determined by a coupled enzyme assay, in which GGT transfers γ-glutamyl group from the substrate L- -Glutamyl *p*-nitroanilide, liberating the chromogen, *p*-nitroanilide, at 418 nm proportional to the GGT present. Lactate dehydrogenase (LDH) in plasma was determined based on the conversion of pyruvate to lactate at 340 nm, alkaline phosphatase (ALP) based on converting nitro phenol phosphate into nitrophenol and phosphate at 405 nm, creatinine phosphokinase (CPK) based on the conversion of creatinine phosphate into creatinine at 340 nm and based on optical density (OD) absorption and the formula presented in the kits' manual [14].

Statistical Analysis

A significant difference in biochemical characteristics of fish exposed to different concentrations of paraquat was examined using one-way ANOVA. Data were examined for normality (Kolmogorov-Smirnov test). The significant means were compared by Duncan's test and a $P < 0.05$ was considered statistically significant. Statistical analyses were performed using SPSS 19 (IBM) software (Chicago, IL, USA). Data were presented as mean ± SD.

RESULTS

No mortality was observed during the experiment. Alterations in the blood biochemical parameters are presented in table 1. Compared

with the control, a significantly increase was observed in AST activity in the plasma of fish exposed to 0.4 $mg.L^{-1}$ paraquat on day 21. Paraquat caused a significant increase in the plasma ALT and LDH activities.

Although a significant increase was found in ALP activity in blood of common carp exposed to 0.2$mg.L^{-1}$ paraquat, the fish exposed to 0.4 $mg.L^{-1}$ paraquat exhibited a significant decrease in ALP activity. CPK activity showed an overall increase in experimental groups of fish. A significant decrease in GGT activity was observed in fish exposed to sub-lethal concentrations of paraquat.

Glucose levels significantly increased on day 21 in the plasma of the fish which were exposed to paraquat. Fish exposed to sub-lethal concentrations of paraquat showed a significant decrease in total protein, albumin and globulin levels. Cholesterol and triglyceride levels in blood of fish exposed to both concentrations of paraquat for 21 days significantly decreased as compared with their levels in plasma of the control group. Our results also showed a significant increase in plasma creatinine levels in fish exposed to both concentrations of paraquat at the end of the period when compared with the control group.

DISCUSSION

The main objective of this study was to investigate the effects of sub-lethal concentrations of paraquat on the blood biochemical parameters of common carp to determine the risks associated with the application of, and the subsequent leaching of this herbicide from agricultural farms into surface waters. AST, ALT, LDH, ALP, GGT and CPK are found in almost all cells of the body tissues, such as heart, kidneys, liver, skeletal muscles, brain, erythrocyte, intestine and gills. The release of intercellular enzymes into the blood and their increased activity in plasma are the most important clinical signs in the diagnosis of damage to cell membranes [15].

Our results indicated a significant increase in AST activity in the fish exposed to high concentration of paraquat may be due to the cellular toxicity. Paraquat or its metabolites might be indirectly caused oxidative stress and damage the liver cells [2]. In the present study, increased ALT activity was observed in plasma of fish exposed to paraquat, which might be due to increased cell membrane permeability or cell membrane damage of hepatocytes. However, decreased activity of GGT and ALP (at high concentration levels of paraquat) occurs either via direct action of paraquat on the activity of these enzymes or inhibition of their biosynthesis [16]. Moreover, a lower than normal GGT and ALP activity may occur as a result of reduced synthesis and severe tissue damage [17]. A decreased ALP activity may be due to malnutrition with low protein assimilation. Ablood cell hemolysis and an increase in blood magnesium ion can inhibit ALP activity [18].

Table 1. Alterations in the blood biochemical parameters of common carp, *Cprinus carpio* exposed to sub-lethal concentrations of paraquat.

Blood biochemical parameters	Sub-lethal concentrations of paraquat		
	Control	0.2 mg L^{-1} Paraquat	0.4 mg L^{-1} Paraquat
AST (U L^{-1})	44.22±3.77[a]	47.13±8.67[a]	57.24±3.94[b]
ALT (U L^{-1})	14.67±1.91[a]	30.80±7.33[b]	30.47±4.72[b]
LDH (U L^{-1})	146.94±11.34[a]	259.45±78.17[b]	267.17±75.24[b]
ALP (U L^{-1})	59.33±5.80[b]	98.64±17.39[c]	38.60±2.64[a]
CPK (U L^{-1})	318.90±76.30[a]	1007.29±88.53[c]	817.15±130.23[b]
GGT (U L^{-1})	14.15±1.06[b]	5.27±0.89[a]	4.29±0.78[a]
Glucose (mg dL^{-1})	58.32±9.76[a]	85.14±10.63[b]	96.88±10.87[c]
Total protein (g dL^{-1})	4.06±0.35[b]	2.64±0.33[a]	2.92±0.23[a]
Albumin (g dL^{-1})	2.03±0.28[b]	1.28±0.25[a]	1.49±0.17[a]
Globulins (g dL^{-1})	2.03±0.53[b]	1.36±0.24[a]	1.42±0.35[a]
Cholesterol (mg dL^{-1})	145.03±16.86[b]	62.77±3.15[a]	55.67±10.95[a]
Triglycerides (mg dL^{-1})	222.85±16.62[c]	126.45±16.22[b]	96.24±19.68[a]
Creatinine (mg dL^{-1})	0.14±0.02[a]	1.12±0.25[b]	1.18±0.20[b]

- Significant differences between values when compared with control groups were characterized by alphabet symbol (P<0.05). Values represent mean S.D

The increased LDH activity caused by paraquat could be explained by changes in the cellular metabolism process during the treatment. Paraquat significantly increased LDH activity in the plasma of common carp, which indicated a serious liver damage. S Increased lipid peroxidation and reduced cellular total antioxidant capacity are the most important factors in increasing the fragility of the cell membrane in fish exposed to paraquat [2].

CPK has been found in high concentrations in cellular cytoplasm of cardiac and skeletal muscles of fish [19]. The increased activity of CPK may be indicative of a disorder in muscle fibres. A similar result was found in common carp exposed to diazinon [15], chlorpyrifos [20], malthion [21], and fenpropathrin [22].

An increase in blood glucose of fish exposed to paraquat may reflect an increased need for energy to counteract the effects of stress caused by paraquat toxicity. Hyperglycemia or elevated blood glucose levels indicate impaired glucose and lipid metabolism and degradation of glycogen in liver [23]. There is strong evidence that exposure to paraquat causes decreased G6PDH activity, degradation of glycogen in liver and muscle tissues and increases blood glucose levels [2]. When exposed to pesticides, *Heteropneustes fossilis* [24], *C. carpio* [25], and *O. mykiss* [15] experienced an increase in blood glucose levels.

Total protein is an important constituent of cells and tissues as it plays a vital role in the physiology of living organisms. There was a decrease in total protein indicating increased mobilization of protein reserves to adapt to stress conditions. Malnutrition, reduced efficiency of the liver in protein synthesis, and reduction of nutrient absorption, especially protein, in the digestive system may be important factors in decreasing plasma total protein. Albumin is the most abundant multifunctional single chain protein in blood plasma. Furthermore, total protein may be reduced due to severe liver and kidney damage. Decreased plasma globulins and albumin levels may be associated with decreases in total protein levels in plasma. Reduced albumin and globulin levels may be due to the effect of paraquat on the biosynthesis of albumin in liver, liver dysfunction, nephritic syndrome and malnutrition [7, 26]. Decreased globulin levels may reduce the resistance of fish to pathogens. Continuous exposure to sub-lethal concentrations of diazinon resulted in significantly decreased levels of total protein, albumin and globulin in rainbow trout [15] and common carp [25] in 30 days.

Results of this investigation of sub-lethal paraquat poisoning in common carp suggest that lipid metabolism is impaired. Decreased cholesterol and triglyceride levels in the blood of fish exposed to paraquat may be attributed to the influence of this herbicide on the synthesis of cholesterol and triglycerides in liver or reduction in the uptake of lipids and fatty acids via intestine. Consumption of cholesterol and triglycerides to provide energy to the cells in order to counter the effects of paraquat poisoning and malnutrition may decrease levels of blood cholesterol and triglycerides.

Any increase in plasma creatinine levels is a biomarker of kidney dysfunction, because creatinine is normally removed from the blood and then excreted from the body [7]. Creatinine levels in plasma reflected the toxicity in the kidney of fish exposed to sub-lethal concentrations of paraquat for 21 days.

CONCLUSION

This study supports the study's hypothesis because sub-lethal concentrations of paraquat had significant effects on the blood biochemical parameters of common carp. Continuous exposure of common carp to sub-lethal concentrations of paraquat leads to blood biochemical alterations and homeostasis disruption.

ACKNOWLEDGMENTS

This study was supported by grant from Behbahan Khatam Al-anbia University of Technology. The authors are grateful to Maryam Banaee for proofreading the manuscript. The authors declare that there are no conflicts of interest.

REFERENCES

1. Banaee M, Davoodi M, Zoheiri F. Histopathological changes induced by paraquat on some tissues of gourami fish (Trichogaster trichopterus). Open Vet Sci J 2013;3(1):36-42.
2. Sharifinasab Z, Banaee M, Mohiseni M, Noori A. Vitamin C and Chitosan Alleviate Toxic Effects of Paraquat on Some Biochemical Parameters in Hepatocytes of Common Carp. Iran J Toxicol 2016;10(1):31-40.
3. Tukmechi A, Rezaee J, Nejati V, Sheikhzadeh N. Effect of acute and chronic toxicity of paraquat on immune system and growth performance in

rainbow trout, Oncorhynchus mykiss. Aquacult Res 2014;45(11):1737-43.
4. Ma J, Li Y, Niu D, Li Y, Li X. Immunological effects of paraquat on common carp, Cyprinus carpio L. Fish Shellfish Immunol 2014;37(1):166-72.
5. Khalighi Z, Rahmani A, Cheraghi J, Ahmadi MRH, Soleimannejad K, Asadollahi R, et al. Perfluorocarbon attenuates inflammatory cytokines, oxidative stress and histopathologic changes in paraquat-induced acute lung injury in rats. Environ Toxicol Phar 2016;42:9-15.
6. Wunnapuk K, Liu X, Peake P, Gobe G, Endre Z, Grice JE, et al. Renal biomarkers predict nephrotoxicity after paraquat. Toxicology letters. 2013;222(3):280-8.
7. Banaee M. Physiological dysfunction in fish after insecticides exposure.In: Insecticides often undesired but still so Important. Edited by StanislavTrdan. InTech. 2013.p.103-42.
8. Mobasher M, Aramesh K, Aldavoud S, Ashrafganjooei N, Divsalar K, Phillips C, et al. Proposing a national ethical framework for animal research in Iran. Iran J Public Health 2008;37(1):39-46.
9. Deivasigamani S. Effect of herbicides on fish and histological evaluation of common carp (Cyprinus carpio). Int J Appl Res 2015;1(7):437-40.
10. Johnson AM, Rohlfs EM, Silverman LM. Proteins. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B. Saunders Company 1999; 477-540.
11. Sacks DB. Carbohydrates. In: Burtis CA, Ashwood ER, eds. Tietz Textbook of Clinical Chemistry. 3rd ed. W.B. Saunders Company, Philadelphia;1999.p.766-85.
12. Rifai N, Bachorik PS, Albers JJ. Lipids, lipoproteins and apolipoproteins. In: Burtis CA, Ashwood ER, eds. Tietz Textbook of Clinical Chemistry. 3rd ed. W.B. Saunders Company, Philadelphia;1999.p. 809-61.
13. Foster-Swanson A, Swartzentruber M, Roberts P, Feld R, Johnson M, Wong S. Reference interval studies of the rate-blanked creatinine/Jaffe method on BM/Hitachi systems in six US laboratories. Clin Chem. 1994;40(1057).
14. Moss DV, Henderson AR. Clinical enzymology. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B. Saunders Company; 1999.p.617-721.
15. Banaee M, Sureda A, Mirvaghefi A, Ahmadi K. Effects of diazinon on biochemical parameters of blood in rainbow trout (Oncorhynchus mykiss). Pestic Biochem Phys 2011;99(1):1-6.
16. Mehrpak M, Banaee M, Nematdoost Haghi B, Noori A. Protective Effects of Vitamin C and Chitosan against Cadmium-Induced Oxidative Stress in the Liver of Common Carp (Cyprinuscarpio). Iran J Toxicol 2015;9(30):1360-7.
17. Atli G, Ariyurek SY, Kanak EG, Canli M. Alterations in the serum biomarkers belonging to different metabolic systems of fish (Oreochromis niloticus) after Cd and Pb exposures. Environ Toxicol Pharmacol 2015;40(2):508-15.
18. Farah HS, Al-Atoom AA, Shehab GM. Explanation of the decrease in alkaline phosphatase (ALP) activity in hemolysed blood samples from the clinical point of view: In vitro study. Jordan J Biol Sci 2012;5(2):125-8.
19. Banaee M, Mehrpak M, Hagi BBN, Noori A. Amelioration of cadmium-induced changes in biochemical parameters of the muscle of Common Carp (Cyprinus carpio) by Vitamin C and Chitosan. Int J Aqua Biol 2015;3(6):362-71.
20. Banaee M, Haghi BN, Ibrahim ATA. Sub-lethal toxicity of chlorpyrifos on Common carp, Cyprinus carpio (Linnaeus, 1758): Biochemical response. Int J Aqua Biol 2014;1(6):281-8.
21. Banaee M, Sureda A, Shahaf S, Fazilat N. Protective effects of silymarin extract on malthion-induced zebra cichlid (Cichlasoma nigrofasciatum) hepatotoxicity. Iran J Toxicol 2015;9(28):1239-46.
22. Banaee M, Sureda A, Zohiery F, Hagi BN, Garanzini DS. Alterations in biochemical parameters of the freshwater fish, Alburnus mossulensis, exposed to sub-lethal concentrations of Fenpropathrin. Int J Aqua Biol 2014;2(2):58-68.
23. Acker CI, Nogueira CW. Chlorpyrifos acute exposure induces hyperglycemia and hyperlipidemia in rats. Chemosphere 2012;89(5):602-8.
24. Saha S, Kaviraj A. Effects of cypermethrin on some biochemical parameters and its amelioration through dietary supplementation of ascorbic acid in freshwater catfish Heteropneustes fossilis. Chemosphere. 2009;74(9):1254-9.
25. Banaee M, Mirvagefei A, Rafei G, Amiri BM. Effect of sub-lethal diazinon concentrations on blood plasma biochemistry. Int J Environ Res 2008; 2(2): 189-98.
26. Ahmad E, Rabbani G, Zaidi N, Ahmad B, Khan RH. Pollutant-induced modulation in conformation -lactamase activity of human serum albumin. PLos One. 2012;7(6):e38372-3.