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Original Article

SUB-LETHAL TOXICITY OF POTASSIUM CYANIDE ON NILE TILAPIA (*OREOCHROMIS NILOTICUS*): BIOCHEMICAL RESPONSE

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ABSTRACT

Objective: The present study was carried out to investigate the influences of sublethal toxicity of potassium cyanide (KCN) exposure to *Oreochromis niloticus* with special reference to blood biochemistry.

Methods: Three groups of fish (25 in each group) were treated with different concentrations of 0 (control), 0.1 and 0.2 mg/L KCN for 2 weeks. The Blood samples were drawn after 2 weeks of exposure and serum biochemical analysis including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), gamma-glutamyl transferase (GGT), glucose, triglyceride, cholesterol, creatinine, total protein, and albumin activities were measured.

Results: KCN exposure at both concentrations caused significant ($P<0.05$) elevation of AST and LDH activities and creatinine concentration as compared to the control. Indeed, ALT, ALP and glucose levels in the fish treated with 0.2 mg/L KCN were significantly higher than those levels from controls. On the other hand, the biochemical parameters including GGT, triglyceride, cholesterol, total protein, and albumin did not change significantly following cyanide exposure.

Conclusion: The current study has clearly indicated the alterations in some of the measured serum biochemical parameters which reflect tissue damages, especially in liver and kidney, due to chronic cyanide intoxication in *Oreochromis niloticus* and may be used for better understanding the pathophysiology of this toxicity and as an aid in the diagnosis of cyanide poisoning in fishes.

Keywords: Nile tilapia, *Oreochromis niloticus,* Cyanide toxicity, Serum biochemical profile.

INTRODUCTION

Aquatic vertebrates live in an intimate contact with the environment, making them more susceptible to pollutants. Among aquatic species, fish are the major target for the action of toxicants. Fishes are largely being used for the assessment of the quality of an aquatic environment and as such can serve as bioindicators of environmental pollution [1]. The primary site of action of cyanide is presumed to be the central nervous system (CNS) [2]. Cyanide inhibits the mitochondrial enzyme cytochrome oxidase in the respiratory electron transport chain of the mitochondria, impairing both oxidative metabolism and the associated process of oxidative phosphorylation [3]. Additionally, a number of other enzymatic processes are also inhibited which exacerbate the cyanide toxicity [1].

Cyanide is one of the most toxic chemical substances on Earth. The extreme toxicity of cyanide arises from its readiness to react with other elements and hence interfere with normal biological processes [4]. The sensitivity of aquatic organisms to cyanide is highly species specific, and also influenced by water pH, temperature and oxygen content, as well as the life stage and conditions of the organism. Fish and aquatic invertebrates are particularly sensitive to cyanide exposure [5]. Cyanide is a potent and rapid-acting asphyxiant; it induces tissue anoxia through inactivation of cytochrome oxidase, causing cytotoxic hypoxia in the presence of normal hemoglobin oxygenation. The effect of the hypoxia causes depression of the central nervous system that can result in respiratory arrest and leading to death [6].

The use of cyanide compounds in mining industries, coupled with limitations in the current analysis and monitoring of these compounds, raises serious concerns regarding public safety and environmental protection at mine sites using cyanide processing. Cyanide and its salts are used extensively in electroplating, extraction of ores (Silver and gold), metal processing, photographic processes, production of synthetic rubber, chemical synthesis, manufacturing of plastic, pesticides, dehairing of hides, laboratory processes, manufacturing of dyes and pigments [7-9] and are also found in water and food consumed by animals and humans. Chronic exposure to low levels of cyanide is suspected to be responsible for various neuropathic and thyrotoxic conditions in humans. However, cyanide also tends to react readily with many other chemical elements, and is known to form, at a minimum, hundreds of different compounds [10]. Many of these breakdowns compounds, while generally less toxic than the original cyanide, are known to be toxic to aquatic organisms. In addition, they may persist in the environment for long periods of time, and there are evidences that some forms of these compounds could be accumulated in plants [11] and fish tissues [12]. The Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) is a widely cultured species because it grows and reproduces in a wide range of environmental conditions and tolerates stress induced by handling [13]. The fish is currently ranked second only to carps in global production [14]. Previously, tilapia was consumed mainly in Africa and Asia but nowadays it has been touted as the "new white fish" replacing the depleted ocean stocks of cod and heck, leading to a worldwide demand for the fish [15]. The toxicity of cyanide to fish can be influenced by a variety of factors including concentration, environmental temperature, dissolved oxygen content, pre-exposure and age [16]. Although a number of studies have investigated the effects of sublethal cyanide exposure on the serum biochemical parameters in various animal species [4, 17-21], very little is known about fish species. Thus, the objective of the present study was to investigate the effects of sublethal exposure to potassium cyanide (KCN) on serum biochemical changes in *Oreochromis niloticus*.

MATERIALS AND METHODS

Collection of experimental fish

The Nile tilapia (*Oreochromis niloticus*; total n=75), weighing 60±10 g, were obtained from the Fisheries Department, Anantapur, Andhra

Pradesh, India. Fish was acclimatized for 7 days before the commencement of the experiment and were fed daily with commercial fish feed at 2.5% total body weight at a fixed time. Physicochemical conditions of the water during the experimental period were dissolved oxygen, 5.8–6.3 ppm; temperature, 25±2°C; pH, 7.1±0.5. Photoperiod was a 12:12 light–dark cycle. The water in aquariums was renewed every 24 h.

Experimental design

After acclimation the *Oreochromis niloticus* fish were divided randomly into three groups of 25 each and held in three glass aquaria, each containing 250 L fresh water. Experimental groups were exposed either to 0 (control), 0.1 and 0.2 ppm potassium cyanide (Sigma-Aldrich Pty. Ltd. 12 Anella Avenue, Castle hill nsw 2154, Australia) for 14 days. At the end of each exposure, 15 fish starved for 24 h were collected randomly from each aquarium and anesthetized in diluted tricaine methanesulfonate. Blood samples (approximately 0.8 mL) were collected from the caudal vena. Blood serum separation was done by centrifugation at 750 g for 20 min and serum samples were stored at −80 ℃ further analysis.

Biochemical assays

Serum biochemical analysis including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), gamma-glutamyl transferase (GGT), glucose, triglyceride, cholesterol, creatinine, total protein, and albumin were done using commercial colorimetric kits (Cayman chemicals-USA).

Statistical analysis

All calculations were performed using SPSS/PC software. All results were analyzed using one way analysis of variance (ANOVA), followed by Duncan's multiple comparisons test. The level of significance was set at $P<0.05$.

RESULTS

The effects of KCN exposure on the values (mean ± SEM) of the measured biochemical parameters in serum of *Oreochromis niloticus* are presented in table 1. As shown in table 1, KCN exposure at both concentrations caused significant ($P<0.05$) elevation of AST and LDH activities and creatinine concentration as compared to controls. Indeed, ALT, ALP and glucose levels in the *Oreochromis niloticus* treated with 0.2 ppm KCN were significantly higher than those levels from control. On the other hand, serum levels of other measured biochemical variable including GGT, triglyceride, cholesterol, total protein, and albumin did not change significantly following KCN exposure as compared to control (table 1).

DISCUSSION

Fish is a heterogeneous group of aquatic animals. Since the fish are ectothermic aquatic vertebrates, having economic values as natural resources of proteins and other fish products, their physiologic, pathologic and toxicologic studies are emerging fields at the present days. Such fields pertaining to fish physiology, pathology and toxicology are further worth attention due to the significance of fish in human nutrition, as people of almost every nation agree to include fish in their normal diet as a rich source of quality protein [22]. It is informed that important elements responsible for normal physiology of animals and their intact structure of various organs for physiological life sustaining processes are hematopieotic tissue, hematological parameters, blood serum biochemistry, lipid profile and circulating blood [23].

The toxic substances cause fluctuations in hematological parameters, either by enhancing their number or concentration by promoting their biosynthetic activities or their fall in number or concentration by suppressing their biosynthetic sites [24]. In clinical and veterinary medical sciences, the hematological parameters are considered as good health indicators of animals as well as for environment [25].

Cyanide is one of the most toxic chemical to fish, as fish are one thousand times more sensitive to cyanide than human [26]. This active sensitivity of fish to cyanide therefore makes fish an excellent biomarker for the presence of cyanide in water [27]. Pollution of the aquatic ecosystem stresses the animals and disturbs their metabolism by altering the enzyme activity, damage and dysfunction the tissues and hindering growth all that associated with biochemical changes [28]. Analysis of biochemical parameters could help to identify the target organs of toxicity as well as the general health status of animals. It may also provide an early warning signal in stressed organism [29, 30].

Table 1: Serum biochemical parameters in *Oreochromis niloticus* exposed to different concentrations of potassium cyanide (KCN) for two weeks (n=15 in each group)

Parameter	Control	KCN 0.1 mg/L	KCN 0.2 mg/L
AST (U/L)	109.75±6.89 [a]	161.39±12.63 [b]	172.21±12.03[b]
ALT (U/L)	39.03±4.11 [a]	51.29±5.17[a,b]	58.25±4.39[b]
ALP (U/L)	18.25±0.69 [a]	22.18±1.17 [a]	27.47±1.21 [b]
GGT (U/L)	2.13±0.08	2.27±0.16	2.01±0.15
LDH (U/L)	395.68±12.15 [a]	472.19±8.19 [b]	460.35±9.89 [b]
Glucose (mg/dL)	50.89±2.25 [a]	51.83±2.41[a]	59.65±3.17 [b]
Triglyceride (mg/dL)	153.75±11.04	131.15±12.09	151.19±16.01
Cholesterol (mg/dL)	181.36±17.45	162.34±30.41	168.01±12.05
Total protein (g/dL)	3.25±0.17	3.17±0.21	4.09±0.35
Albumin (g/dL)	1.65±0.25	1.81±0.18	1.30±22.01
Creatinine (mg/dL)	0.49±0.07 [a]	0.74±0.08 [b]	0.85±0.06 [b]

a,b mean±SEM in each row with no common superscript differ significantly ($P<0.05$)

Transaminases are important enzymes known to play a key role in mobilizing L-amino acids for gluconeogenesis and function as links between carbohydrate and protein metabolism under altered physiological, pathological conditions [30, 31]. Increase in the levels of these enzymes in liver, muscle and gills of fishes can be considered as a response to the stress induced by cyanide to generate ketoacid-like ketoglutarate and oxaloacetate for contributing to gluconeogenesis and/or energy production necessary to meet the excess energy demand under the toxic manifestation [32]. In the present study, AST and ALT levels were found to be increased following cyanide exposure at both concentrations, although the increase was not significant for ALT activity at 0.1 mg/L KCN. In agreement with these results, [33]showed significant increases of AST and ALT enzyme activities in rats drinking water contaminated with cyanide. Similar findings were also observed by Naveed et al., [34] in *C. punctatus.* Agrahari et al., [35] observed an increase in the ALT and AST of the catfish exposed to pesticide and opined that this increase in the activity of these enzymes is an indicator of cellular damage.

Indeed, ALT activities in pigs treated with 6.0 mg/kg of KCN were significantly increased [21]. On the other hand, there was no statistically significant difference in the serum activities of AST in rabbits [17] and both AST and ALT activities in goats [20] and rats

[36] following cyanide administration. These differences could be due to variation in the dose, duration of exposure, and the species. The increased activities of AST and ALT in the present study would indicate the deleterious hepatic and renal damages of cyanide exposure in *Oreochromis niloticus* that is reminiscent of previously reported pathological findings in liver and kidney of several animal species and humans exposed to cyanide [4, 36, 37].

Tissue distribution of alkaline phosphatase is virtually ubiquitous especially within cell membranes and would easily leak out of the cell in cyanide-induced tissue damage [17]. Elevation of serum ALP has been also reported due to liver damage from poisoning by some chemicals in humans [38]. In the present study serum ALP activity was increased significantly following exposure to 0.2 ppm KCN as compared to control. In agreement with this finding, increased serum alkaline phosphatase has been reported in the rabbits fed mash plus cyanide compared to controls fed mash alone [4, 17]. Significant increases in serum ALP and ALT activities in addition to histopathological changes in liver, lung and kidney tissues has been also reported following chronic cyanide intoxication in rabbits [19].

LDH, the terminal enzyme in vertebrate anaerobic glycolysis, is one of the enzymes that have been employed for diagnosing liver, muscle, and gill damages caused by pollutants in fish [39]. Increased activity of LDH is a characteristic feature of a shift from aerobic to anaerobic metabolism leading to an elevated rate of pyruvate conversion into lactate, resulting in lactic acidosis [40]. In the present study, significant rise in LDH activity was observed in cyanide-exposed *Oreochromis niloticus* compared to control group. Increases in serum and tissue levels of LDH activities are characteristic features of lactic acidosis resulting from the inhibitory effect of cyanide on aerobic metabolism [19] and increased LDH activity in liver of *Cyprinus carpio* was reported by Kamalaveni et al., [41]. Similarly, increased LDH activity in freshwater fish by arsenic treatment was reported by Shobha Rani et al., [42].

Cyanide is also known to alter glucose metabolism [43]. There are some literatures available that report diabetes as a toxic effect produced by ingesting cassava, a cyanogenic plant, in various species [44-47]. Based on the present results, cyanide exposure at the dose of 0.2 mg/L caused the significant increase in glucose concentration in *Oreochromis niloticus* that is reminiscent to the results reported previously in swine and rats [48, 36]. On the other hand, no alterations in plasma glucose were observed following chronic cyanide exposure in goats, rats and rabbits [17, 18, 20].

Based on the result of the present study, increased creatinine concentration was observed following cyanide exposure that might be associated with kidney damage due to cyanide exposure. In line with this finding significant increases in serum creatinine concentrations have been reported following KCN administration in rats [33] and pigs [21]. Okolie and Osagie, have reported that degenerative changes in the kidney sections of the cyanide-fed rabbits may be responsible for the significant increases in serum urea and creatinine [4].

CONCLUSION

The observed alterations in some measured serum biochemical parameters would reflect tissue damages, especially in liver and kidney, due to chronic cyanide intoxication in *Oreochromis niloticus* and may be used for better understanding the pathophysiology of this toxicity and as an aid in the diagnosis of cyanide poisoning in fish species.

CONFLICT OF INTEREST

The authors declare that we have no conflict of interest.

REFERENCES

1. Prashanth MS, Neelagund SE. Free cyanide-induced Biochemical changes in Nitrogen metabolism of the Indian major carp, *Cirrhinus mrigala*. J Basic Clin Physiol Pharmacol 2007;8(4):77-287.
2. Okolie NP, Audu K. Correlation between cyanideinduced decreases in ocular Ca2+-ATPase and lenticular opacification. J Biomed Sci 2004;3(1):37-4.
3. Daya S, Walker RB, Anoopkumar-Dukie S. Cyanide-induced free radical production and lipid peroxidation in rat brain homogenate is reduced by aspirin. Metab Brain Dis 2000;15(3):203-10.
4. Okolie NP, Osagie AU. Liver and kidney lesions and associated enzyme changes induced in rabbits by chronic cyanide exposure. Food Chem Toxicol 1999;37:745-50.
5. Dube PN, Shwetha A, Hosetti BB. Effect of exposure to sublethal concentrations of sodium cyanide on the carbohydrate metabolism of the Indian Major Carp *Labeo rohita* (Hamilton, 1822). Pesqui Vet Bras 2013;33(7):914-9.
6. Shwetha A, Hosetti BB. Acute effects of zinc cyanide on the behaviour and oxygen consumption of the Indian major carp, *Cirrhinus mrigala*. World J Zoology 2009;4(3):238-46.
7. Agency for toxic substances and disease registry (ATSDR), Toxicological profile for cyanide. Draft for public comment. Prepared for the U. S. Public Health Service by Technical Resources, Inc., under Contract No. 68-03-3268. Revised by Syracuse Research Corporation under Contract No. 68-03-3521. Oak Ridge National Laboratory; 1988.
8. US. Environmental protection agency. Cyanide health advisory (draft). Office of Drinking Water; 1985.
9. World Health Organization, Cyanide. In: Guidelines for drinking-water quality. Vol. 2. Health criteria and other supporting information. Geneva. 1984. p. 97.
10. Flynn CM, Haslem SM. Cyanide chemistry-precious metals processing and waste treatment: US. Bur Mines Inf Circ 1995;9429:282.
11. Eisler R. Cyanide Hazards to Fish, Wildlife, and Invertebrate: A Synoptic Review: Contaminant Hazard Review report 23, US. Dept. Interior, Fish and Wildlife Service; 1991. p. 55.
12. Heming T, Thurston RV, Meyn EL, Zajdel R. Acute toxicity of thiocyanate to trout. Trans Am Fish Soc 1985;114:895-905.
13. Tsadik GG, Bart AN. Effects of feeding, stocking density and water-flow rate on fecundity, spawning frequency and egg quality of Nile tilapia, *Oreochromis niloticus* (L.). Aquacult 2007;272:380-8.
14. Ridha MT. Comparative study of growth performance of three strains of Nile tilapia, *Oreochromis niloticus*, L. at two stocking densities. Aquacult Res 2006;37:172-9.
15. Yue YR, Zhou QC. Effect of replacing soybean meal with cottonseed meal on growth, feed utilization and hematological indexes for juvenile hybrid tilapia, *Oreochromis niloticus* x *O. aureus*. Aquacult 2008;284:185-9.
16. Ballantyne B. Toxicology of cyanides. In: Clinical and experimental toxicology of cyanides. B Ballantyne, TC Marrs, Eds. IOP Pub. Ltd, Bristol; 1987. p. 41-126.
17. Okolie NP, Osagie AU. Differential effects of chronic cyanide intoxication on heart, lung and pancreatic tissues. Food Chem Toxicol 2000;38(6):543-8.
18. Soto-Blanco B, Marioka PC, Gorniak SL. Effects of long-term low-dose cyanide administration to rats. Ecotoxicol Environ Saf 2002;53(1):37-41.
19. Okolie NP, Iroanya CU. Some histologic and biochemical evidence for mitigation of cyanide-induced tissue lesions by antioxidant vitamin administration in rabbits. Food Chem Toxicol 2003;41:463-9.
20. Soto-Blanco B, Gorniak SL. Milk transfer of cyanide and thiocyanate: cyanide exposure by lactation in goats. Vet Res 2003;34:213-20.
21. Manzano H, Sousa AB, Soto-Blanco B, Guerra JL, Maiorka PC, Gorniak SL. Effects of long-term cyanide ingestion by pigs. Vet Res Commun 2007;31:93-104.
22. Saksena DK. Biomonitoring of Pb, Ni, Cr, Hg with the help of bryophytes in Nainital. In: V Nath, Bishen Singh, Mahenra Pal Singh, Ed. Proceeding of National Conference on Bryology; 1999. p. 159-76.
23. Davis JRE. Molecular and cell biology. In: Mechanisms of disease, Tomlinson S. Heagerty AM, Weetman AP, eds. Cambridge University Press: Cambridge, UK; 1997. p. 13-62.
24. Scott GR, Sloman KA. The effects of environmental pollutants on complex fish behaviour: integrative behavioural and physiological indicators of toxicity. Aquat Toxicol 2004;68:369-92.
25. Schuett DA, Lehmann J, Goerlich R, Hamers R. Hematology of swordtail, Xiphiphorus helleri. 1: Blood parameters and light microscopy of blood cells. J Appl Ichthyol 1997;13(2):83-9.

26. Hosetti BB, Dube PN. Evaluation of acute toxicity of copper cyanide to freshwater fish, *Catla catla* (Hamilton). J Central Eur Agric 2010;12(1):135-44.
27. Adeyemo OK. Haematological and histopathological effects of Cassava Mill Effluent in *Clarias gariepinus.* Afr J Biomed Res 2005;8(3):179-83.
28. Osman AGM, Abdel Reheem ABM, Abuelfadl KY, Gadelrab AG. Enzymatic and histopathologic biomarkers as indicators of aquatic pollution in fishes. Nat Sci 2010;2(11):1302-11.
29. David M, Ramesh H, Patil VK, Marigoudar SR, Chebbi SG. Sodium cyanide-induced modulations in the activities of some oxidative enzymes and metabolites in the fingerlings of *C. carpio* (L). Toxicol Environ Chem 2010;92:1841-9.
30. Prashanth MS. Acute toxicity, behavioral and nitrogen metabolism changes of sodium cyanide affected on tissues of *Tilapia mossambica* (Perters). Drug Chem Toxicol 2012;35(2):178-83.
31. Shwetha A, Hosetti BB, Dube PN. Toxic effects of zinc cyanide on some protein metabolites in freshwater fish, *Cirrhinus mrigala* (Hamilton). Int J Environ Res 2012;6(3):769-78.
32. Okafor PN, Anoruo K, Bonire AO, Maduagwu EN. The role of low-protein and cassava-cyanide intake in the aetiology of tropical pancreatitis. Global J Pharmacol 2008;2(1):6-10.
33. Elsaid FG, Elkomy MM. Aqueous garlic extract and sodium thiosulphate as antidotes for cyanide intoxication in Albino rats. Res J Medicine Med Sci 2006;1(2):50-6.
34. Naveed A, Janaiah C, Venkateshwarlu P. The effects of lihocin toxicity on Protein metabolism of the fresh water edible fish, *Channa punctatus* (Bloch). Toxicol Environ Health Sci 2010;3(1):18-23.
35. Agrahari S, Pandey KC, Gopal K. Biochemical alteration induced by monocrotophos in the blood plasma of fish, *Channa punctatus* (Bloch). Pesticide Biochem Physiol 2007;88:268-72.
36. Tulsawani RK, Debnath M, Pant SC, Kumar O, Prakash AO, Vijayaraghavan R, *et al*. Effect of sub-acute oral cyanide administration in rats: Protective efficacy of alpha-ketoglutarate and sodium thiosulfate. Chem-Biol Interact 2005;156:1-12.
37. Sousa AB, Soto-Blanco B, Guerra JL, Kimura ET, Gorniak SL. Does prolonged oral exposure to cyanide promote hepatotoxicity and nephrotoxicity? Toxicol 2002;174:87-95.
38. Bogusz M. The usefulness of enzymatic tests in acute poisoning. Arch Toxicol 1975;34:159-67.
39. Neff JM. Use of biochemical measurements to detect pollutant mediated damage to fish. In: Aquatic toxicology and hazard assessment: seventh symposium. RD Cardwell, R Purdy, RC Bahner, Eds. ASTM STP 854, ASTM, Philadelphia; 1985. p. 155-83.
40. Abdel-Hameid NAH. A protective effect of calcium carbonate against arsenic toxicity of the Nile catfish, *Clarias gariepinus*. Turkish J Fisheries Aquatic Sci 2009;9:191-200.
41. Kamalaveni K, Gopal V, Sampson U, Aruna D. Recycling and utilization of metabolic wastes for energy production is an index of biochemical adaptation of fish under environmental pollution stress. Environmental Monitoring and Assessment 2003;86:255-64.
42. Shobha Rani A, Sudharsan R, Reddy TN, Reddy PUM, Raju TN. Alternations in the levels of dehydrogenases in a freshwater fish, *Tilapia mossambica* (Peters) exposed to arsenic toxicity. Indian J Environ Health 2000;42:130-3.
43. Way JL. Cyanide intoxication and its mechanism of antagonism. Ann Rev Pharmacol Toxicol 1984;24:451-81.
44. Kamalu BP. The effect of a nutritionally-balanced cassava (*Manihot esculenta,* Crantz) diet on endocrine function using the dog as a model.1. Pancreas. Br J Nutr 1991;65:365-72.
45. Geldof AA, Becking JL, de Vries CD. Histopathological changes in rat pancreas after fasting and cassava feeding. *In Vivo* 1992;6:545-51.
46. Akanji AO, Famuyiwa OO. The effects of chronic cassava consumption, cyanide intoxication and protein malnutrition on glucose tolerance in growing rats. Br J Nutr 1993;69:269-76.
47. Petersen JM. Tropical pancreatitis. J Clin Gastroenterol 2002;35:61-6.
48. Jackson LC. Behavioral effects of chronic sublethal dietary cyanide in an animal model: implications for humans consuming cassava (*Manihot esculenta*). Hum Biol 1988;60:597-614.