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Original Research Article

Biochemical Alterations Induced by Sublethal Cyanide Exposure in Common Carp *(Cyprinus carpio)*

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ABSTRACT

The present study was designed to study the effects of sublethal cyanide exposure on some blood biochemical parameters in common carp. Three groups of fish (25 in each group) were treated with different concentrations of 0 (control), 0.1 or 0.2 mg/L potassium cyanide (KCN) for 2 weeks. At the end of each exposure, blood samples were collected and serum biochemical parameters were measured using validated methods. Cyanide exposure at both concentrations caused significant (P<0.05) elevation of aspartate aminotransferase and lactate dehydrogenase activities and creatinine concentration as compared to control group. Indeed, alanine aminotransferase, alkaline phosphatase and glucose levels in the carp treated with 0.2 mg/L KCN were significantly higher than those levels from controls. On the other hand, other measured biochemical parameters including gamma-glutamyl transferase, triglyceride, cholesterol, total protein, and albumin did not change significantly following cyanide exposure. The observed alterations in some measured serum biochemical parameters would reflect tissue damages, especially in liver and kidney, due to chronic cyanide intoxication in common carp 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.

Key words: *Cyprinus carpio*, serum biochemical profile, cyanide poisoning

INTRODUCTION

The discharge of toxic pollutants into waterways may result in acute or chronic toxicity in fish species. Cyanides are one of the major classes of toxic chemicals of concern for aquatic biota in certain waste-receiving waters (Heath 1995). Cyanide may be produced by toxic gases during the pyrolysis of plastic or nitrile-based polymer fibres, by extracts of plants containing cyanogenic glycosides (e.g. cassava) or from industrial waste (e.g. electroplating). Massive death of fish and other aquatic biota due to the accidental discharges of cyanide wastes have been reported (Eisler and Wiemeyer 2004) and freshwater fish are the most cyanide-sensitive group of aquatic organisms tested. However, various sensitivities to cyanide have been reported in different fishes (Dzombak *et al.* 2005; Akinsiku *et al.* 2010).

The toxicity of cyanide derives mainly from its potency as a respiratory poison in aerobic organisms. This toxicant is readily absorbed across gill membranes in fish and is a potent, rapid acting asphyxiant inducing tissue anoxia and cytotoxic hypoxia due to inhibition of cytochrome oxidase (Eisler and Wiemeyer 2004). In addition to acute cyanide poisoning, chronic toxicity of cyanide has frequently been reported in recent years, and it is suggested that the most widespread problems arising from cyanide are from chronic dietary, industrial and environmental sources (Mathangi and Namasivayam 2000). Experimental studies on different animal species have shown that prolonged sublethal cyanide exposure can induce pathologic effects on various tissues (Dixon and Leduc 1981; Okolie and Osagie 1999; Soto-Blanco *et al.* 2001; Sousa *et al.* 2002; Manzano *et al.* 2007).

The metabolism of cyanide and its main metabolite, thiocyanate, is species-linked, and toxicokinetic parameters of cyanide compounds vary in different species (Sousa *et al.* 2003). 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 (Ballantyne 1987). Although a number of studies have investigated the effects of sublethal cyanide exposure on the serum biochemical parameters in various animal species (Okolie and Osagie 1999; Okolie and Osagie 2000; Soto-Blanco *et al.* 2002; Okolie and Iroanya 2003; Soto-Blanco and Gorniak 2003; Manzano *et al.* 2007), very little is known about fish species. Thus, the present study was conducted to

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investigate the effects of direct sublethal exposure to potassium cyanide (KCN) on serum biochemical profile in common carp.

MATERIALS AND METHODS

Experimental design and sampling

Common carp (*C. carpio*; total n=75), weighing 60±10 g, were obtained from a local commercial farm. They were divided randomly into three groups of 25 each and held in three glass aquaria, each containing 250 l fresh water. Fish were 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, 5.5–6 ppm; temperature, 25±1°C; pH, 7±0.5. Photoperiod was a 12:12 light–dark cycle. The water in the aquariums was renewed every 24 h. Experimental groups were exposed either to 0 (control), 0.1, or 0.2 ppm potassium cyanide (Merck, Darmstadt, Germany) 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 MS-222. 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 −70 °C until analysis.

Biochemical assays and analysis

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 (Pars Azmoon, Iran).

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

Table 1. Serum biochemical parameters in carp exposed to different doses 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)	111.87±7.75[a]	164.43±13.77[b]	175±12.10[b]
ALT (U/L)	41.07±4.42[a]	54.67±5.54[a, b]	60.48±4.98[b]
ALP (U/L)	19.47±0.73[a]	23.25±1.21[a]	28.63±1.36[b]
GGT (U/L)	2.25±0.10	2.64±0.20	2.07±0.19
LDH (U/L)	412.93±12.44[a]	496.27±8.51[b]	482.67±10.02[b]
Glucose (mg/dL)	51.94±2.32[a]	52.90±2.58[a]	62.57±3.68[b]
Triglyceride (mg/dL)	154.98±11.21	126.18±12.38	152.58±16.16
Cholesterol (mg/dL)	184.57±17.87	160.40±30.28	169.05±12.15
Total protein (g/dL)	3.27±0.19	3.20±0.25	4.14±0.40
Albumin (g/dL)	1.72±0.27	1.88±0.21	1.28±.22
Creatinine (mg/dL)	0.47±0.06[a]	0.76±0.09[b]	0.87±0.07[b]

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

DISCUSSION

Biochemical analysis is a fundamental tool used to diagnose and predict the outcome of diseases and to monitor the effects of therapeutic, nutritional, toxic and environmental factors in human and veterinary medicine. The effects of chronic cyanide toxicity on the growth, metabolism, reproduction, and histopathology of freshwater fish has been investigated (Koenst *et al.* 1977; Lind *et al.* 1977; Kimball *et al.* 1978; Dixon and Leduc 1981) and the results of the present work indicate that sublethal cyanide exposure led to alterations in some blood biochemical parameters that may help to determine mode of action of toxicant and organ dysfunction following cyanide exposure.

In toxicological studies, the alterations in the enzymatic activities directly reflect the metabolic disturbances and cell damage in specific organs (Casillas *et al.* 1983). The response to pollution is reflected as changes in some enzyme activities that might provide a tool for specific early warning sign for aquatic pollution (Velmurugan *et al.* 2008). 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, Elsaid and Elkomy (2006) showed significant increases of AST and ALT enzyme activities in rats drinking water contaminated with cyanide. Indeed, ALT activities from pigs treated with 6.0 mg/kg of KCN were significantly increased (Manzano *et al.* 2007). On the other hand, there was no statistically significant difference in the serum activities of AST in rabbits (Okolie and Osagie 2000) and both AST and ALT activities in goats (Soto-Blanco and Gorniak 2003) and rats (Tulsawani *et al.* 2005) 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 deleterious hepatic and renal damages of cyanide exposure in common carp that is reminiscent of previously reported pathological findings in liver and kidney of several animal species and humans exposed to cyanide (Okolie and Osagie 1999, Sousa *et al.* 2002, Tulsawani *et al.* 2005). It has been also reported that focular degeneration in the kidneys and liver was partly correlated with the altered activities of ALT and AST in rats exposed to cyanide for 15 d (Sousa *et al.* 2002). Moreover, some cyanide-induced histopathological changes, including degenerative hepatic necrosis and loss of the characteristic chord-like pattern of the hepatocytes have been also reported in rainbow trout (Dixon and Leduc 1981).

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 (Okolie and Osagie 2000). Elevation of serum ALP has been also reported due to liver damage from poisoning by some chemicals in humans (Bogusz 1975). In the present study serum ALP activity was increased significantly following exposure to 0.2 ppm KCN as compared to control group. 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 (Okolie and Osagie 1999;

Okolie and Osagie 2000). Significant increases in serum ALP and ALT activities in addition to histopathological derangements in liver, lung and kidney tissues has been also reported following chronic cyanide intoxication in rabbits (Okolie and Iroanya 2003).

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 (Neff 1985). In the present study, significant rise in LDH activity was observed in cyanide-exposed common carp 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 (Okolie and Osagie 1999; Okolie and Iroanya 2003).

Cyanide is also known to alter glucose metabolism (Way 1984). There are some works that report diabetes as a toxic effect produced by ingesting cassava, a cyanogenic plant, in various species (Kamalu 1991; Geldof *et al.* 1992; Akanji and Famuyiwa 1993; Petersen 2002). Based on the present results, cyanide exposure at the dose of 0.2 mg/L caused significant increase in glucose concentration in common carp that is reminiscent to the results reported previously in swine and rats (Jackson 1988; Tulsawani 2005). On the other hand, no alterations in plasma glucose were observed following chronic cyanide exposure in goats, rats and rabbits (Okolie and Osagie 2000, Soto-Blanco *et al.* 2002, Soto-Blanco and Gorniak 2003).

Based on the present study results, 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 (Elsaid and Elkomy 2006) and pigs (Manzano *et al.* 2007). It has been also 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 observed in these animals (Okolie and Osagie 1999).

In 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 common carp 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.

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