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Effect of Sodium Cyanide Toxicity on Health and Growth of *Oreochromis niloticus*

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ABSTRACT

A total number of 290 *Oreochromis niloticus (O. niloticus)* fingerlings with an average body weight 10 ± 0.2g were used to determine acute and chronic toxicity of sodium cyanide (NaCN) and their effect on health and growth of *O. niloticus.*

The results revealed that, 96hrs LC_{50} of NaCN for *O. niloticus* fingerlings was 0.75 mg/L. The exposed fish showed abnormal and vigorous circular movement followed by intervals of slow horizontal movement, respiratory disorder, the fish did not respond to tested reflexes, some fish showed blindness , liver was dark red friable, gills and kidney were congested. Fish exposed to $^1/_{10}$ (0.08mg/L) LC_{50} of NaCN showed significant decrease in growth performance, a while significant increase of some hematological and biochemical parameters were recorded. A significant decrease of gamma globulin was recorded in *O. niloticus* fingerlings exposed to $^1/_{10}$ and $^1/_{20}$ LC_{50} of NaCN.

INTRODUCTION

Cyanide is commonly used in countries such as the Philippines, Indonesia and Papua new Guinea for mining gold and silver and also in poisoning pests in fish farm ponds. Cyanide is widely used by fishermen to poison live fish to facilitate capture specifically those in coral reefs. This practice was first reported in the Philippines in 1962 , where fish collector named Gonzales, squirt sodium cyanide to stun ornamental fish on the reefs *(1).*

The water contaminated with cyanide come from different industrial processes such as manufacturing synthetic fiber, coal conversion. Wastes or cooking effluents come from the iron and steel industries, electroplating wastes, petrochemical wastes and in mining operation. After extraction and recovery of metals substantial the amount of cyanide delivered to tailings pond will create environmental problems due to toxicity of cyanide *(2).*

Cyanide exists in three forms in waste water , free cyanide such as hydrogen cyanide , simple cyanide such as sodium cyanide and complex cyanide such as iron and nickel cyanide *(2).* Biomass burning is known to be major and perhaps dominant source of hydrogen cyanide and acetonitril to the atmosphere. Burning also may liberate cyanide from cyanogenic glucosides which are present at substantial concentrations in many plants *(3).* Cyanide concentration in runoff from burned areas was much higher than unburned area. Free cyanide concentration in runoff average 49µg/L equal to LC_{50} of rainbow trout *(4).*

The present study was planned to determine acute and chronic toxicity of NaCN and their effect on health and growth of *O. niloticus.*

MATERIAL AND METHODS

A total number of 290 *O. niloticus* fingerlings with an average body weight 10±0.2g were used. Fish were collected from Abbassah Fish Hatchery, Sharkia Province. They were apparently healthy and free from any external lesions. Fish were kept in a glass aquaria provided with aerator for 15 days for acclimatization before start of experiments.

Glass aquaria were used. Each aquarium (60 x50x30cm) provided with aerator and thermostatically controlled heater. The aquaria were filled with clean and dechlorinated water.

The fish were fed on based diet contain crude protein 34%. The amount of feed (on dry mather basis) given daily to fish was 10% of body weight and the fish were fed 3 times daily.

Sodium cyanide (NaCN) with molecular weight 49.01g/mol was used. It is white solid inorganic compound soluble in water, produced by BDH company for chemical (England). It is also called cyanogran.

Determination of 96 hrs LC_{50} of NaCN (acute toxicity) in *O. niloticus* fingerlings

A total of 200 *O. niloticus* fingerlings were divided into 20 equal groups. All groups were exposed to different concentrations of NaCN (0.0-1.0mg/L) for 96hrs (Table 1). The 96 hrs LC_{50} was estimated ***(5)***. Evaluation of general health condition of fish, clinical signs, post mortem lesions and mortality were recorded ***(6,7)***. Histopathological examination of tested fish was performed ***(8)***.

Table 1. Preliminary trials for zero and hundred % mortality of *O. niloticus* exposed to different concentrations of NaCN .

Group n=10`	Concentration of NaCN (mg/L)	Mortality during 96 hrs				Total mortality	Total mortality %
		1st day	2nd day	3rd day	4th day		
1	0 (control)	0	0	0	0	0	0
2	0.10	0	0	0	0	0	0
3	0.15	0	0	0	0	0	0
4	0.20	0	0	0	0	0	0
5	0.25	0	0	0	0	0	0
6	0.30	0	0	0	0	0	0
7	0.35	0	0	0	0	0	0
8	0.40	0	0	0	0	0	0
9	0.45	0	0	0	0	0	0
10	0.50	0	0	0	0	0	0
11	0.55	0	0	0	0	0	0
12	0.60	1	1	0	0	2	20
13	0.65	2	1	0	1	4	40
14	0.70	2	1	1	0	4	40
15	0.75	2	1	2	0	5	50
16	0.80	4	2	1	0	7	70
17	0.85	4	2	1	0	7	70
18	0.90	7	2	0	0	9	90
19	0.95	9	0	0	0	9	90
20	1.00	10	0	0	0	10	100

The effect of $^{1}/_{10}$ and $^{1}/_{20}$ 96 hrs LC_{50} of NaCN on health and growth of *O. niloticus* fingerlings

A total of 90 *O. niloticus* fingerlings were divided into 3 groups , each group had two replicates with 15 fish density in each aquarium. The first and second groups were exposed to $^{1}/_{10}$ and $^{1}/_{20}$ of 96 hrs LC_{50} of NaCN respectively while the third group was kept as control . The experiment was kept for 8 weeks.

Growth performance , body gain *(9)* , body gain % *(10)* and food conversion ratio (FCR) *(9)* were determined.

Blood samples for haematological and biochemical analysis were collected from the caudal blood vessels *(6).* Blood cells (RBCs and WBCs) counts were carried out *(11, 12).* Haematocrite packed cell volume (PCV) was measured *(13)* . Haemoglobin concentration (Hb) was performed *(14, 15).* Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined colourimetrically *(16, 17).* Serum creatinine, *(18)* and Serum urea *(19)* were determined. Serum cortisol level was determined using radioimmunoassay *(20).*

Humoral immune response, total serum proteins were determined using biuret reaction *(21).*

The obtained data was statistically analysed using analysis of variance procedure *(22)* .

RESULTS AND DISCUSSION

Acute toxicity of NaCN in *O. niloticus* fingerlings

The actual estimation of 96 hrs. LC_{50} of NaCN in *O. niloticus* fingerlings was 0.75 mg/L with 100% mortality at 1.0 mg/L (Table 2). Fish exposed to high doses of NaCN showed abnormal and vigorous circular movement followed by intervals of slow horizontal movement, respiratory disorder , the fish did not respond to tested reflexes and some fish showed blindness (Fig. 1-A). Liver was dark red and friable in consistency . Gills and Kidney were congested with slimy gills. Similar findings were previously recorded *(23, 24)* . These may be attributed to cyanide cause direct inhibition of cytochrome oxidase enzyme which has important role in the respiratory chain resulting in cellular hypoxia and cytotoxic anoxia that is potentially fatal. Also cyanide toxicity associated with neuropathies, hypothyroidism and depression in central nervous system *(25)* .

The liver of *O. niloticus* exposed to acute toxicity of NaCN revealed severe hydropic degeneration, mild vacuolations in the cytoplasm of some hepatocytes, small multifocal areas of coagulative necrosis, congestion of the hepatic blood vessels and sinusoid and aggregation of leuckocytes mainly lymphocytes in portal areas (Fig. 1-B). The epithelial covering of the gill arch showed vacuolated cells, gill filaments were focally displaced with leukocytes mainly lymphocytes, congestion of the bronchial blood vessels , hemorrhages and edema among the supporting fibrous tissues of secondary lamellae (Fig. 1-C). Our results are consistent with the previously cited investigation *(26).*

Effect of $^{1}/_{10}$ (0.08mg/L) and $^{1}/_{20}$ (0.04mg/L) 96 hrs. LC_{50} of NaCN on health and growth of *O. niloticus* fingerlings

The results demonstrated in Table 3 revealed that, fish exposed to $^{1}/_{10}$ 96 hrs LC_{50} of NaCN for 8 weeks showed significant decrease in final body weight , body gain , body gain % and increase of feed conversion ratio. Nearly similar results were previously obtained *(27, 28).* These results may be due to the adverse effect of cyanide as stress factor on fish.

Fish exposed to $^{1}/_{10}$ 96hrs LC_{50} of NaCN for 8 weeks showed significant increase in Hb, PCV, RBCs, WBCs, AST, ALT , urea, creatinine and cortisol (Table 4) . Nearly similar results were previously obtained *(29)* . The increase in Hb, PCV and RBCs may be attributed to increase synthesis of RBCs to overcome the inhibition of cytochrome oxidase enzyme caused by cyanide. Also, the increase of cortisol may be due to the stress of

cyanide on fish. In addition, the increase of ALT and AST may be due to liver damage or increasing protein metabolism.

Fish exposed to $^{1}/_{10}$ and $^{1}/_{20}$ 96hrs LC_{50} of NaCN for 8 weeks revealed a significant decrease in gamma globulin (Table 5). These may be due to degeneration of liver and kidney. Our results were supported by previously obtained results. Necrosis and hepatic damage occurs in fish exposed to 10 mg /L hydrogen cyanide for 9 days *(26)* . Acute nitrate toxicity of fish result in significant decrease of gamma globulin *(30).*

Table 2. Estimation of 96 hrs LC_{50} of NaCN in *O. niloticus* fingerlings.

Group	Concentration of NaCN (mg/L)	Total number of dead fish at 96 hrs	A	B	a x b	∑ a x b
I	0.55	0				
II	0.60	2	0.05	1	0.05	
III	0.65	4	0.05	3	0.15	
IV	0.70	4	0.05	4	0.20	
V	0.75	5	0.05	4.5	0.22	
VI	0.80	7	0.05	6	0.30	
VII	0.85	7	0.05	7	0.35	
VIII	0.90	9	0.05	8	0.40	
IX	0.95	9	0.05	9	0.45	
X	1.00	10	0.05	9.5	0.47	
						2.59

96 hrs LC_{50} = Highest dose - $\frac{\sum a \times b}{n}$

$= 1 - \frac{2.59}{10} = 1 - 0.25 = 0.75$ mg/L NaCN

Where:

a. Constant factor between two successive doses

b. The mean of dead fish in each group.

n. The number of fish in each group.

$\sum$ (ax b) = sum of a x b

Table 3. Effect of $^{1}/_{10}$ and $^{1}/_{20}$ 96 hrs LC_{50} of NaCN on growth performance of *O. niloticus* fingerlings.

Group and dose / Parameter	1 Control	2 $^{1}/_{10}$ 96 hrs. LC_{50} of NaCN (0.08mg/l)	3 $^{1}/_{20}$ 96 hrs. LC_{50} of NaCN (0.04mg/l)
Initial body weight (g)	12.5 ± 0.28^{a}	12.4 ± 0.34^{a}	12.6 ± 0.34^{a}
Final body weight (g)	20.5 ± 0.4^{a}	17.6 ± 0.5^{b}	19.9 ± 0.49^{a}
Body gain (g)	8 ± 0.84^{a}	5.2 ± 0.23^{b}	7.3 ± 0.17^{a}
Body gain (%)	64 ± 1.7^{a}	41.9 ± 0.86^{c}	57.9 ± 0.83^{b}
Feed intake (g)	25.2 ± 1.7^{a}	21.3 ± 0.51^{a}	25.5 ± 0.9^{a}
Feed conversion ratio	3.15 ± 0.06^{c}	4.09 ± 0.06^{a}	3.49 ± 0.06^{b}

Means within the same raw carrying different superscripts are significant ($P\leq0.05$)

Table 4. Effect of $^{1}/_{10}$ and $^{1}/_{20}$ 96 hrs LC_{50} of NaCN on some hematological and biochemical parameters of *O. niloticus* fingerlings.

Parameter \ Group and dose	1 Control	2 $^{1}/_{10}$ 96 hrs. LC_{50} of NaCN (0.08mg/l)	3 $^{1}/_{20}$ 96 hrs. LC_{50} of NaCN (0.04mg/l)
Hb (g/dL)	6.1±0.23^{a}	6.8±0.34^{a}	6.2±0.23^{a}
PCV (%)	24.5±0.63^{b}	30±1.1^{a}	25±1.7^{b}
RBCs (10^{6}/µL)	1.01±0.06^{b}	1.7±0.14^{a}	1.21±0.09^{b}
WBCs (10^{3}/µL)	29±1.4^{b}	75±2.5^{a}	31±1.1^{b}
AST (µ/L)	201±5.77^{b}	260±7.5^{a}	250±8.08^{a}
ALT (µ/L)	45±1.7^{b}	72±9.7^{a}	69±2.3^{a}
Urea (mg/dL)	5±0.28^{b}	7±0.57^{a}	6±0.23^{ab}
Creatinin (mg/dL)	0.22±0.03^{b}	0.32±0.04^{a}	0.31±0.04^{a}
Cortisol (nmol)	3.5±0.57^{c}	26±1.44^{a}	21±1.7^{b}

Means within the same raw carrying different superscripts are significant ($P \leq 0.05$)

Table 5. Effect of $^{1}/_{10}$ and $^{1}/_{20}$ 96 hrs LC_{50} of NaCN on humoral immunity of *O. niloticus* fingerlings

Parameter (g/dL) \ Group and dose	1 Control	2 $^{1}/_{10}$ 96 hrs. LC_{50} of NaCN (0.08mg/l)	3 $^{1}/_{20}$ 96 hrs. LC_{50} of NaCN (0.04mg/l)
Albumin	2.512±0.03^{c}	2.980±0.02^{b}	3.212±0.02^{a}
Alpha globulin	0.592±0.09^{c}	0.670±0.06^{b}	0.720±0.03^{a}
Beta globulin	0.870±0.01^{b}	0.920±0.02^{ab}	0.950±0.01^{a}
Gamma globulin	0.860±0.07^{a}	0.650±0.01^{b}	0.590±0.01^{c}
Total protein	4.834±0.06^{c}	5.22±0.06^{b}	5.472±0.07^{a}

Means within the same raw carrying different superscripts are significant ($P \leq 0.05$)

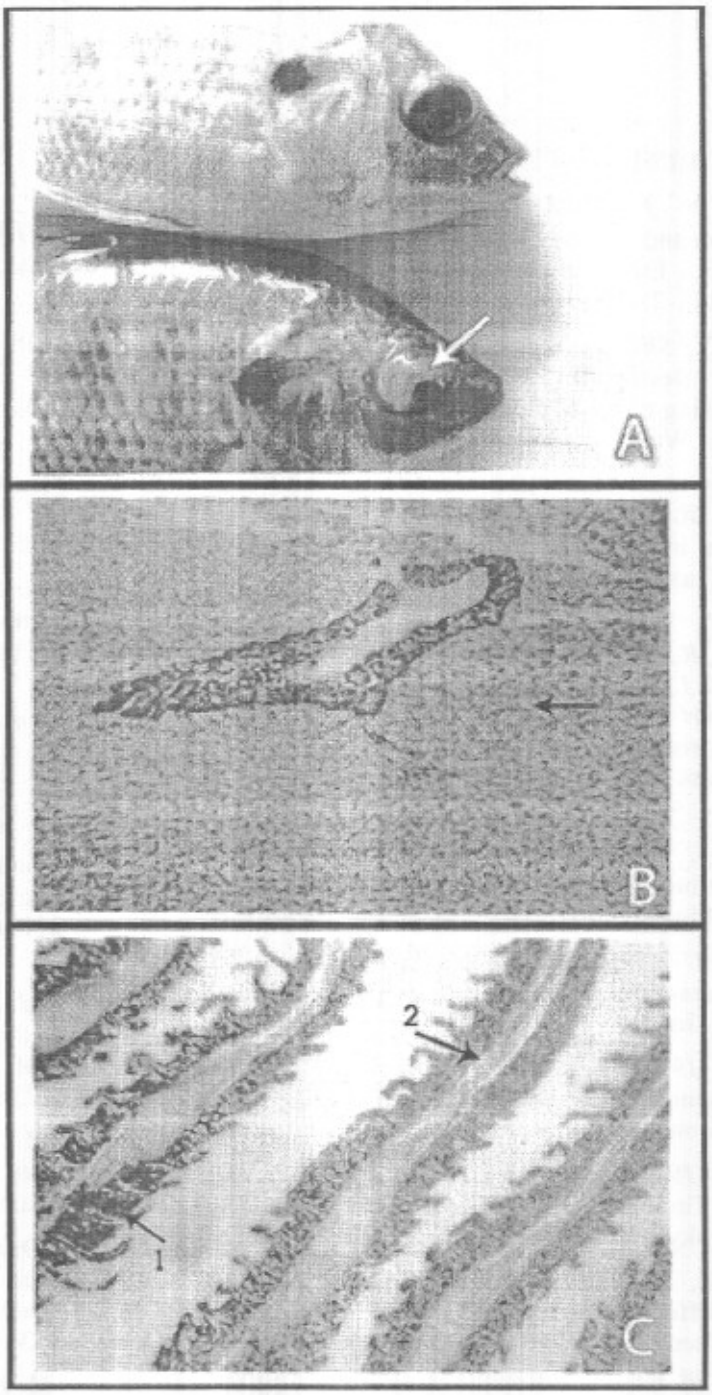

Fig. 1. Effect of acute toxicity of NaCN on *O. niloticus* fingerlings:
(A) showed blindness of *O. niloticus* (arrow). (B) showed hydropic degeneration and vacuolation in the cytoplasm of some hepatocytes (arrow) H & E x 300. (C) show focal displacement of gill filaments and lamellae with lymphocytes (arrow 1) and edema among the supporting fibrous connective tissue (arrow 2) , H & E x 300.

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الملخص العربى

تأثير سمية سيانيد الصوديوم على صحة ونمو البلطى النيلى

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كلية الطب البيطرى – جامعة الزقازيق

أستخدم عدد ٢٩٠ من أصبعيات البلطى النيلى بمتوسط وزن ٠,٢ ± ١٠ جرام لتحديد التسمم الحاد والمزمن لسيانيد الصوديوم وتأثيرهما على صحة ونمو البلطى النيلى.

أوضحت نتائج الدراسة مايلى:

١- أن الجرعة نصف المميته لسيانيد الصوديوم بعد ٩٦ ساعة فى إصبعيات البلطى النيلى هى ٠,٧٥ ملجم/ لتر.

٢- أظهرت الأسماك المعرضة لتركيزات عالية من سيانيد الصوديوم حركة عصبية عنيفة فى دوائر يتخللها أوقات من الحركة البطيئة وظهور بعض الأعراض التنفسية مع عدم أستجابة الأسماك للمؤثرات الخارجية وظهور بعض حالات العمى . تلون الكبد باللون الأحمر الداكن مع أحتقان شديد فى الخياشيم والكليتين .

٣- أن الأسماك المعرضة لتركيز ٠,٠٨ ملجم /لتر وهى تعادل ١/١٠ من الجرعة نصف المميته لسيانيد الصوديوم بعد ٩٦ ساعة أظهرت انخفاض ملحوظ فى قياسات النمو بينما كان هناك زيادة ملحوظة فى التحليل الكيميائى ومكونات الدم.

٤-وجد أن هناك أنخفاض ملحوظ فى الجاما جلوبيولين فى الأسماك المعرضة لتركيزات ١/١٠ ، ١/٢٠ من الجرعة نصف المميته لسيانيد الصوديوم بعد ٩٦ ساعة .