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RESEARCH ARTICLE

BEHAVIOURAL AND HISTOPATHOLOGICAL EFFECTS OF CHLORINE ON NINE TILAPIA FISH, *OREOCHROMIUS NILOTICUS*

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ABSTRACT

Tilapia fish, *oreochromius niloticus* were exposed to different sublethal concentrations (30mgll and 50 mg/l) of chlorine to observe behavioral alterations and histopathological alterations in liver. The fishes showed marked changes in their behavior when exposed to the test solution of different concentrations. The fishes showed rapid swimming than in control, hyperactivity, loss of balance, increased surfacing activity. The lives showed marked alterations, the focal areas of necrosis vacuolation and dilation and thrombosis formation in central vein, rupture in the hepatocytes, Cell damage, Moreover, pyknosis (PY) and hemorrhages was seen.

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INTRODUCTION

Fish is an excellent source of protein in human diet. The unique feature which differentiates fish food other animal protein sources is the presence of omega-3 fatty acids (Lee and Reasner, 2000 and Lemos *et al*., 2005). Chlorine is a chemical element with symbol (Cl) and atomic number 17. It has a relative atomic mass of about 35.5, Chlorine is in the halogen group (17) and is the second lightest halogen, following fluorine. The element is a yellow-green gas under standard conditions, where it forms diatomic molecules. Chlorine has the highest electron affinity and the third highest electro negativity of all the reactive elements. For this reason, chlorine is a strong oxidizing agent. Free chlorine is rare on Earth, and is usually a result of direct or indirect oxidation by oxygen. The most common compound of chlorine, sodium chlorine (common salt), has been known since ancient time .Around 1630, chlorine gas was first synthesized in a chemical reaction, but not recongnized as a fundamentally important substance. The chemistry of chlorine in natural waters is complex (White, 1972). Toxicological studies have primarily concentrated on the two forms which are apparently the most toxic to aquatic life (Heath, 1977). The first form, commonly referred to as free chlorine, is the portion of chlorine injected into the water that remains as molecular chlorine (Cl_2), hypochlorous acid (HOCl), or a hypochlorite ion (OCl–) after chlorine demand has been satisfied. The second form, called monochloramine or combined chlorine (NH_2Cl), is the portion of chlorine injected into the water that remains combined with ammonia or nitrogenous compounds. After the chlorine demand has been satisfied. The sum of the free and combined forms generally is referred to as total residual chlorine (TRC). The relative proportion of free and combined chlorine present following a chlorination dose depends primarily on the concentration of nitrogenous materials in the water. The chlorine demand is defined as the difference between the amount of chlorine added to the water and the amount of TRC that remains at the end of a specified period (Mattice and Zittel, 1976).

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Objectives

In the present work, an attempt was made to evaluate the short term exposure. Effects of chlorine on the behavior and histology of liver of the freshwater fish *oreochromius nilotics.*

MATERIALS AND METHODS

Materials

Fish: Adult freshwater fish Tilapia (Family: Cichlidae), were obtained from the Hatchery in Sudan University of science and

Technology, Department of fisheries and wildlife science. A total of 23 adult fish of both sexes were used. The average weight of the fish was 90-20 g. the fish were placed in large tanks (34 x 22 x 18 cm) with Aerated tap water and were fed with commercially pellets. Fish were acclimatized for 2 days and an average temperature of 29°C. The tap water used for the experiment had a pH value of 6-7 and the water was tested for chlorine founds.

Chlorine was added as a powder.

In two dosages

- The first group was 30 mg/l with two replicates each of 5fish
- The second group was 50 mg/l with two replicates each 5fish. The third was the control group

Preparation of Stock Solution

Chlorine was procured from the DAL group. Ltd, Bahre Sudan. Stock solution was prepared by dissolving the chlorine powder and diluted with tap water.

Methods

The fish were exposed to dissolved chlorine and record the observations tell fishes were dead. Any dead fish was dissected for its and liver.

Water quality parameters

Temperature (C^o)

Water temperature was measured using an ordinary centigrade measuring thermometer.

PH

Water pH was determined by taking water sample and measuring its pH by portable digital Ph meter

Behavioural Manifestation

The observations of the fish behavior were records during the experiment.

Histopathological Examination

Tissue specimens from fish of all groups were taken from (livers) and fixed in 10 % buffered neutral formalin. Then processed using the normal histological techniques to obtain five micron thick paraffin sections then stained with Hematoxylin and Eosin and examined under light microscope.

RESULTS

Mortality

No mortality was observed in the control group however, mortality increased with the increase in the concentration and the exposure duration of the chlorine. The concentration at which there the highest percent mortality was 50 mg/L and hundred percent mortality were caused in the both concentrations.

Table 1. Shows the fish mortality time exposed to different dosages of chlorine

Concentration Cl_2- (mg $l)_2$	Cumulative mortality				
	Time of exposition				
	5 minute	15 minute	25 minute	35 minute	45 minute
0.0 (control group)	0	0	0	0	0
30	0	2	3	3	2
50	2	3	4	1	0

Behavioural Manifestation

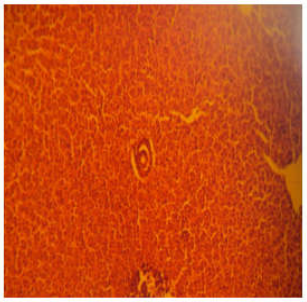
The behavior and condition of the fishes in both the control and test solutions was noted all the time up to mortality complete. The fishes showed marked changes in their behavior when exposed to the test solution of different concentrations. In both concentration of chlorine (30 mg/L, 50 mg/ L) the fishes showed rapid swimming than in control. Behavioural manifestations of acute toxicity like hyperactivity, loss of balance, rapid swimming, increased surfacing activity

Fig 1. Scattered shoal

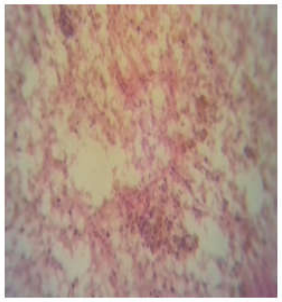
Fig 2. Vertical swimming

Fig 3. Loss of balance

Histopathological Results



(a)



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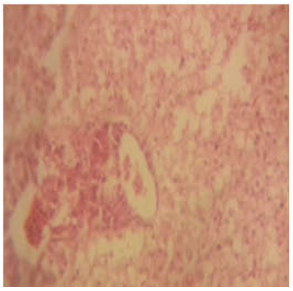

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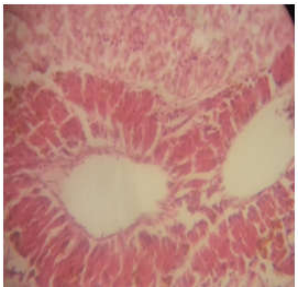

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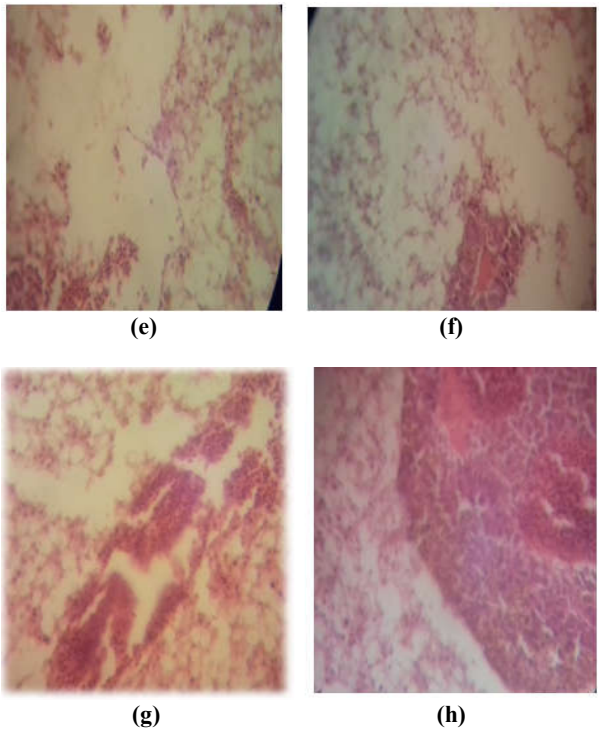

(e) **(f)**

(g) **(h)**

Liver of fish showing the normal (a)(H&E X 200), Focal areas of necrosis(FN)(H&E X200)(b), Dilation and thrombosis formation in central vain (CV). H&E X200(c) and (d), ruptured hepatocytes (RH) and vacuolation (V) H&E X200 (e) and (f), cell damage (CD).H&E X200 (g), pyknosis (PY) and hemorrhage. H&E X400(h)

Table 2. The main types of histopathological changes detected in *Oreochromis niloticus* exposed to chlorine

Organ	*Histopathological changes*	Number of fish in which the effect was detected	
		30 ppm	50 ppm
Liver	Focal areas of necrosis	90%	100%
	Dilation and thrombosis formation in central vein	100%	100%
	Ruptured hepatocytes and vacuolation	80%	100%
	Cell damage	100%	100%
	pyknosis (PY) and hemorrhages	90%	90%

DISCUSSION

Behavioral changes are the most sensitive indication of potential toxic effects studied. In the control group the behavioral and the swimming patterns of the fishes were normal and there was no mortality. In the initial period of exposure to chlorine, the fish stayed motionless and settled to the bottom. This may be attributed to the fact that, the sudden shock caused by the toxicant. The fishes behavioral response started appearing after 5 minute of treatment. The shoaling behaviour was disrupted in the first minutes itself and they were spread out and appeared to be swimming independent of one another.

The disturbance in the shoaling behaviour of the fish in the treated media indicates the loss of group hydrodynamic effect of fish (Zuyev and Bolyayen, 1970) increased swimming activity and entails high expenditure of energy (Zuyev and Bolyayen, 1970). Erratic swimming of the treated fish indicates the loss of equilibrium. Chlorine has profound effect on the central nervous system. The liver showed congestion of central vein and nuclear pyknosis in the majority of hepatic cells. These findings were apparent as the liver considered the organ of detoxification, excretion and binding proteins such as metallothionein (MTs). The cell damages. Similar results were observed by Van Dyk (2003) and Mela *et al.* (2007). Liver of fish is sensitive to environmental contaminants because many contaminants tend to accumulate in the liver and exposing it to a much higher levels than in the environment or other Organs (Heath 1995). Pandey *et al*. (1994) described the alterations in liver and intestine of *Liza parsia* exposed to Hg Cl_2 (0.2 mg Hg l– 1) for 15 days. Similarly (Oliveira Ribeiro *et al*., 2002) reported serious injuries in liver of *Salvelinus alpinus* exposed to 0.15 mg Hg l– 1.

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