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Histological Changes in the Gills of *Oreochroms niloticus* Exposed to Promethazine Hydrochloride

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Abstract: The effect of sub lethal concentration of promethazine hydrochloride (phenergan) on gills of *Oreochromis niloticus* (mean weight and length, 20 g and 12.13 cm, respectively) was investigated under static bioassay for 96 h. The fish treated with promethazine hydrochloride showed abnormal behaviour characterized eratic movement, loss of reflex and hyperventilation during the period of exposure. The LC_{50} of phenergan for *O. niloticus* was determined to be 0.035 g L^{-1}. Histological analysis of the gills showed pathological changes in sub lethal levels tested, caused detachment of epithelial in the primary and secondary lamella. Osmoregulatory and respiratory weakness brought about mucus accumulation couple with epithelia detachment was suggested to cause fish mortality after the exposure to promethazine hydrochloride.

Key words: Promethazine hydrochloride, *Oreochromis niloticus*, gill, histology, phenergan

INTRODUCTION

Man has used prothazine hydrochloride as anaesthetic and sedative. In aquaculture, anesthetics are mainly used during handling and speculative. In aquaculture, anesthetics are mainly used during handling and transportation of fish is either hatchery or market. The aquarium industries as reported by Gopinath (1990) are totally reliant on life transport to move products to market.

There are limited numbers of anesthesia used in the area of aquaculture and those in use include methlyquinoline phenoxyethanol and Tricaine methane sulphonate (MS222) with the dosage of 0.07, 0.513 mg L^{-1} and 0.1 g L^{-1} of water, respectively (Sado, 1985; Jhingran, 1975). Yulin (1996) reported that these chemical are too expensive, thus the use of phenergan which is common and cheaper than the anesthesia counter parts. Although they are found useful as anesthesia and sedative in man, the correct dosage for fish as anesthesia has not been determined. Therefore, in determining the safer level for its application the acute intoxication of fish has to be determined.

Annune (1992) reported that gill tissues are most sensitive to water pollution. Since gills are the primary site for osmorequlation and respiration they are highly vulnerable to lesions due to their contact with pollutants Benediczky *et al*. (1984) come to conclusion that while some pollutants enter the body, there is evidence that some of them exert their effect on the external surface of the fish especially the gills. Exposure of the Nile Tilapia, *O. niloticus* according to Omoregie *et al*. (1992) to sub lethal concentration of malachacite green lead to severe physiological impairment in the fish after a 10 week exposure period. From the study of Memesoic *et al*. (1985), it was reported that paraquat concentrations of 1 and 10 mL^{-1} increased serum transaminase activity in fish species. This was attributed to serious tissue damage of gills, liver and kidney. Water pollution/contamination induces pathological changes in fish. Bernet *et al*. (1999) noted that as an indicator of exposure to contaminants, histology represents a useful tool to assess the degree of pollution. The description and assessment

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of histological changes in different organs represent a very sensitive and crucial parameter in determining cellular changes that occur in target organs such as gills, liver and gonads (Dutta, 1996).

Histological investigations may therefore prove to be a cost-effective tool to determine the health of fish populations, hence reflecting the health of an entire aquatic ecosystem. According to Brasle and Gonzalez (1996), in order to measure the effects toxicants could have on fish species and subsequently human populations, a histological investigation of the gills may produce meaningful and useful results.

The study of Sado (1985) on the anesthetic effect of quinaldine on of *O. niloticus* showed that fish mortality was noticed at the concentration of 75 ppm.

This study investigate the histology of gills of Nile tilapia *Oreochromis niloticus* juveniles exposed to various sub lethal concentrations of promethazine hydrochloride under laboratory condition.

MATERIALS AND METHODS

Oreochromis niloticus juveniles of average weight 20 g and corresponding standard length of 12.13 cm were purchased life from two hatcheries in Akure (Ondo state). They were transported live in 50 L tank to the Department of Fisheries and Wildlife and Federal University of Technology Akure (FUTA) laboratory for the study, where the experiment was carried in 2004.

The fish were acclimated for 3 days in large glass tank (45×60×30 cm) containing dechlorinated and aerated FUTA bore-hole water at Temperature of 25.85+0.11°C.

Promethazinne hydrochloride tablet were purchased from pharmaceutical shop in Akure (Metado-Dafon pharmacy) with code number Guk/drugs/1/846 and batch number 1312. They were grounded into powdery form to enhance solubility and uniform mixing in the liquid medium (water). Before the acute toxicity test, 100 juveniles of *Oreochromis niloticus* were used for range finding test. his served as preliminary test in order to ascertain the range of acute toxicity of promethazine hydrochloride to *O. niloticus*. The quantities used for the range finding test were 0.05, 0.1, 0.15, 0.20 and 0.25 g of phenergan.

The result from range finding test provided a guide for the concentration range to be used definitive test. This was carried out using ten glass tanks filled with 10 L of water. Five ranging concentrations of phenergan (0.01, 0.02, 0.03, 0.04 and 0.05 g) were prepared in duplicate and each was introduced into the glass tanks together with two replicates of control treatment that devoid of phenergan. A total of 120 *O. niloticus* juveniles were used for the test.

The behavioral pattern of *Orechromis niloticus* were observed after the introduction of the phenergan and the following observation were notice in the test media, discoloration, molting and erratic swimming.

Mortality rate was the criterion used in this study, so the number of dead and the living organisms in each test chamber were counted every 24 h.

The water quality parameters like pH, Dissolved Oxygen (DO_2) temperature and alkanet were recorded in every 24 h until 36 h using method described by APHA (1989).

Sample of the gill tissues were taken from the dead fish for microscopy. Four fish were selected from each group so that time for their individual survival will represented range of recorded survival time for the whole sample.

The sections of the gills of the dead fish were excised by dissecting the fish; these were placed in formalin for 3 days after which they were dehydrated in successive concentrations of ethanol. Thereafter the samples were infiltrated and embedded in paraffin wax. These samples were later mounted on the albumenized glass slides and stained with hemetoxytin and eosin for examination and photomicrographs.

RESULTS

The physico chemical parameters of test solution were observed to be fluctuating during the experiment (Table 1). With higher concentration of the test solution fish displayed initial disturbed summing movement rapid opercula movements and surfacing behaviour indicative of avoidance response. It was observed that at the lower concentration of promethazine hydrochloride solution, fish did not show any obvious changes in behaviours during the experiment. Also there is no mortality in the controls and the fish in this group did not show abnormal behavior. The 96 h median lethal concentration of phenegan and its 95% confidence limit for juveniles of Oreochromis niloticus was 0.035 g L^{-1} (Table 2).

Gills of *Orechromis niloticus* exposed to phenergan exhibited varying degree of epithelia hyperplasia among filaments and among treatments. Examination of the gills of untreated (control) *O. niloticus* juveniles (Fig. 1) revealed normal gill filament consisting of primary lamellae with its arrays of delicate secondary lamellae, primary epithelium and secondary epithelium covering the primary and secondary lamellae, respectively with no vacuolation.

There was no observable change in the gill structure of *O. niloticus* juveniles exposed to low concentration of 0.01 and 0.02 g L^{-1}) of the toxicant. It shows that low concentration of promethazine hydrochloride did not cause any histological damage to the gills of *O. niloticus*. But with higher concentrations (0.04 and 0.05 g L^{-1}) the filaments are reduced in length with no lamella present. Also there is partial degeneration of lamellae and hypertrophy of the filament structure of the gills arch. (Fig. 2 and 3).

Table 1: Water quality parameters obtained during exposure of *Oreochromis niloticus* to promethazine hydrochloride for 96 h

	Promethazine hydrochloride concentration (g L^{-1})					
Paramerters	0.00	0.01	0.02	0.03	0.04	0.05
Temperature (°C)	26.53±1.24	26.50±1.12	26.56±1.14	26.65±1.14	26.75±1.40	27.00±1.29
DO_2 (ppm)	3.06±0.97	3.31±0.66	3.34±0.96	3.40±0.98	2.46±1.55	2.93±1.44
Total hardness (ppm)	127.50±12.06	121.88±22.69	131.63±10.01	133.13±11.69	137.00±13.26	142.20±143
pH	6.98±0.25	6.90±0.64	6.96±0.82	7.08±0.66	7.14±0.62	7.07±0.56

(Mean value±SD)

Table 2: Variation in mortality rate of *O. niloticus* exposed to different concentrations of phenergan

Concentration (g L^{-1})	Mortality (%)
0.00	0
0.01	20
0.02	30
0.03	40
0.04	60
0.05	65

LC_{50} = 0.035 g L^{-1}

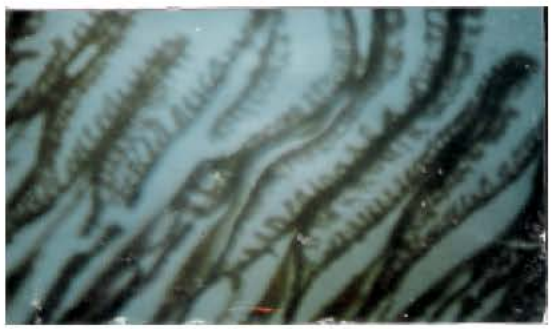

Fig. 1: Gill of *O. niloticus* exposed to 0.00 g L^{-1} (control) of promethazine hydrochloride

Fig. 2: Gill of *O. niloticus* exposed to 0.04 g L^{-1} of promethazine hydrochloride

Fig. 3: Gill of *O. niloticus* exposed to 0.05 g L^{-1} of promethazine hydrochloride

DISCUSSION

The physico chemical parameters of the test solutions fluctuated during the bioassays and this might probably led to the toxic effect of the effluent used. This falls in line with the trends of results reported by Aderiye (1998) that unidentified physico-chemical qualities of the 5% petroleum refinery effluent or precipitated early hatching of African catfish, *Clarias gariepinus* eggs and that hatching percentage was inversely proportioned to the effluent concentration from 25.8% in the control is 2.3% in 100% concentration.

The mortality recorded during the experiment might have come from the alteration in the physico chemical parameters of the media. This observation was similar to the report of Eilien *et al*. (1991) that fish mortality tests are affected by temperature dissolved oxygen concentration, pH and duration of exposure well.

The histological study reveals the adverse effect of the phenergen on vital organ like gill. The examined organ made fragile, the structure as well as their cell deformed. Also the organs were enlarged and porous. The treated fish gills were swollen and the lamella were extensively fused together and congested with blood. This agrees with the work of Aderiye (1998) who reported that the gill structure of the fish *O. niloticus* treated with petrol and engine oil mixture was fused together and that there was an extensive hyperplasia and separation of the epithelia layer from the supportive tissue.

The absence of discernible charge is gill structure of *Oreocromis niloticus* treated with promethazine hydrochloride of two concentrations indicates that fish mortality observed in these levels could have been caused by the accumulation of the mucous on the gills epithelium which might impair osmoregulation and gaseous exchange resulting to suffocation of the fish.

CONCLUSIONS

The study was carried out using Promethazine hydrochloride for toxicity test on Nile Tilapia (*O. niloticus*).The test organisms is very important in warm freshwater captured and cultured fisheries. Physico-chemical parameters (CO_2, pH,Temperature hardness) of the entire test media conducted were within the normal range, so toxicity could not have resulted fro the media physico-chemical parameters. Responses of the test organism in the various concentration of prtomethazine hydrochloride (phenergan) show that the test material could be used as potential toxicant. The LC_{50} value was 0.035 g L^{-1} for fish exposed to prtomethazine hydrochloride (phenergan) for 96 h. The dosage of each concentration and time of exposure influenced fish opercula movement and this increased with increased concentration up till toxic stage when the opercula movement gradually reduced and later stopped.

Finally, the use of prtomethazine hydrochloride as toxicant on *O. niloticus* juveniles is viewed positive therefore this study may be used as basis for toxicity levels for *O. niloticus* juveniles.

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