App. Trop. Agric. Vol 15, Nos 1 & 2, PP 18-21, 2010

Applied Tropical Agriculture

USE OF PROMETHAZINE HYDROCHLORIDE AS ANAESTHETIC AGENT FOR *Clarias gariepinus* (BURCHELL 1822) FINGERLINGS

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Abstract
Anaesthetic test was conducted using values of histological tissues examinations to assess the effects of Clarias gariepinus fingerlings (9.6g) exposure to aqueous extract of promethazine hydrochloride (phenergan) under a static bioassay system. The fishes were exposed to various concentrations of phenergan extracts (50-250mg/l). The time in which each reached anaesthesia decreased with increase in phenergan concentrations. The fingerlings reached anaesthesia in significantly shorter time ($P<0.05$) (10 minutes at 250mg/l concentration) in phenergan but never recovered. All fingerlings exposed to 200mg/l phenergan extract reached anaesthesia within 15 minutes but only 50% of the fish recovered while 50% died within six hours. Fingerlings exposed to 150mg/l phenergan reached anesthesia within 18 minutes and 80% recovered within 2 hours later. It took a longer time (25-40 mins) for fingerlings in the lowest concentrations (50mg/l and 100mg/l) to reach anaesthesia and they all recovered within 30- 60 minutes. As the duration of exposure increased, fish showed increased weakness, they were motionless and gasped for air. The LC_{50} was at 200ml concentration. Excessive mucous secretion was observed. Histological examination showed varying degrees of gill filaments, degeneration in the lamella, cellular infiltration, vacuolation, necrosis and lesion in the gills, liver and skin of the fish. Result of the test provided baseline information for which safe limits of using phenergan as an anaesthetic agent in catfish ponds may be established. The safe margin for C. gariepinus fingerlings (9.6g) is 50-100ml.

Key words: Clarias gariepinus, Promethazine hydrochloride, anaesthesia, histology.

Introduction

Anaesthesia and sedation of fish is very essential to minimize stress and physical damage caused by handling and crowding during research work. There is need to render the fish unconscious or to alleviate pain and if this is not well managed, it could lead to mortality. Apart from minimizing stress, and direct physical injury, sedation is necessary particularly when fishes are transported in bulk over long distances. The use of anaesthetic agents delays many of the normal stress actions. Ross and Ross (1983) noted that benzocaine anaesthesia gave only a mild reduction in oxygen consumption during handling while it has been shown by Houston *et. al.,* (1971) that MS222 (tricaine methane sulphonate) induction produces tranchy-ventilation. General anaesthetic procedures usually act by widespread depression of the central nervous system produced by an action on nerve axons (Winlow *et al.*, 1992). When induction is slow, a series of stages of anesthesia can be observed. This occurs in most animals but this was first described for fish by McFarland (1959). Induction of animals is often accompanied by hyperactivity, ataxia, loss of reflex, no reaction to stimuli or death. The condition of the animals is monitored by maintaining the environment and physiological needs such as oxygen supply and removal of waste gases. There are series of factors which can alter the efficacy of anesthetic processes in fish. These factors can be biological (species, size, lipid content, sex, sexual maturity, body condition, disease status) or environmental (temperature, pH, salinity, mineral content of the environment) (Ross and Geddes, 1979).

Phenergan is a tranquilizer and antihistamine which belongs to the class of phenothiazine derivatives (Goodman and Gildman, 1980). Phenergan is used when sedation is required for a minor procedure or during local anesthesia and it is most useful for reducing anxiety (Bowman *et al.,* 1980). Introduction of phenergan did not cause a loss of consciousness but producesd only a tendency to sleep (Laborit, 1952). According to Ross and Ross (1999), it is preferable that a sedative or anesthetic drug should be effective at low doses and the drug must not produce hyperactivity during induction, it should be water soluble, easy to obtain in bulk, safe to operators and the level of sedation should be easily reversed without prolong ataxia. Generally, synthetic drugs cause death of the fish and destruction of gill epithelial at high doses (Slabber and Morgan, 1982). At very high doses, it induces symptoms of slow suffocation, dizziness, paralysis of gill lamellae and muscle cell depletion in

several freshwater fish (Schweigner, 1957). This study investigates the effects of phenergan on *Clarias gariepinus* fingerlings and the histological changes in their gill and liver.

Materials and Methods

Phenergan tablets were purchased from a pharmaceutical shop, milled and sieved using 0.1mm sieve and 700 g of phenergan powder was dissolved in 2 litres of water at ambient temperature (23°C) for 24 hours, filtered to remove particles and prepare stock solution of 2000 mg/l. This was used for the anaesthesia test in concentrations (50, 100, 150, 200 and 250 mg/l) and introduced into corresponding glass tanks in duplicates and 0.0 mg/l (control). Ten *C. gariepinus* fingerlings (mean wt. 9.6g) were introduced in each of the glass tanks containing 10 litres of water. The time at which each fish reached anaesthesia was recorded. As the fish reached anaesthesia, they were removed from anesthetic agent and introduced into a recovery glass tank containing 20 liters of aerated water to recover. Fish behaviour was monitored in all treatments. The fish were then observed for 12 hours after which the time for recovery was determined. Mean lethal concentration (96h LC_{50}) was calculated from mortality values. Gills and liver were taken for histological examination; the samples were immediately fixed in 10% formaldehyde in labeled bottles. Tissues were processed and embedded in paraffin wax; sections were cut, and stained with haematoxylin and eosin and examined under the microscope. Quantitative data obtained were subjected to one-way analysis of variance (ANOVA) and multiple range tests using SPSS version 11.0 for windows. Relationships were compared using line graph on Microsoft Excel ® 2007.

Results and Discussion

Anaesthesia in the fish increased with increasing concentrations of phenergan and 60% of the fish reached anaesthesia within 40 minutes in the lowest concentration (50mg/l) of aqueous (Fig.1) and they recovered from anesthesia within 30 minutes (Fig.2). In concentrations 100mg/l and 150mg/l, 100% reached anesthesia within 18-25 minutes (Fig.1) but 80% recovered in 150mg/l within 2 hours while 20% recovered to die after eight hours .In the highest concentrations of phenergan (200mg/l and 250mg/l), they all reached anaesthesia within 10-15mins (Fig.1) but they did not recover from anaesthesia, they all died (Table 1, Fig.2). Increasing the anaesthetic dose significantly decreased induction times and recovery time for fish. Similar findings were reported for other fishes by Iversen, et al. (2003), Ross and Ross (2002) and Dong-Won, et al.(2007).

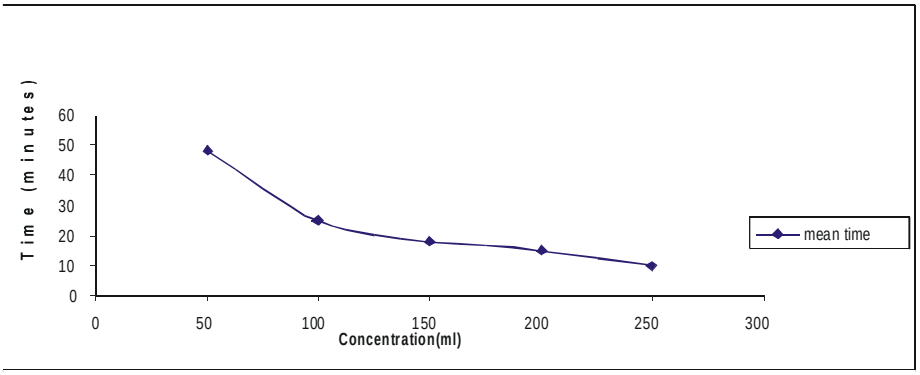

Fig 1. Mean time at which Clarias gariepinus fingerlings reached anaesthesia in varying concentration of P. hydrochloride

Table 1. Anaesthesia of *C. gariepinus* fingerlings exposed to varying concentrations of phenergan

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Concentration anaesthesia (%)	Reached	Time(min)	Recovered(%)	Time(min)	Mortality(%) (ml)
250	100	10	0	-	100
200	100	15	60	132	50
150	100	18	80	120	20
100	100	25	100	60	0
50	60	40	100	30	0

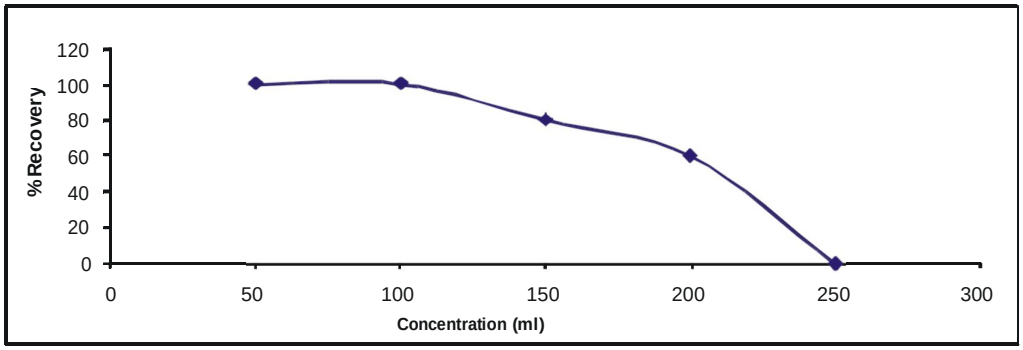

Fig 2. % Recovery of C. gariepinus fingerlings exposed to different concentrations of P. hydrochloride

The 96h LC_{50} occurred at 200ml. Fish exposed to phenergan exhibited increased opercula movement, swimming, hyperactivities and there was a sensitive indicator of physiological stress in fish subjected to high concentrations (200 and 250mg/l) of phenergan (Table 2). Increase in opercula movement is an indication of stress. When phenergan was applied into the water, fish started swimming erratically and moved to the bottom of the tank where they remained motionless (stage of anaesthesia). This observation was similarly reported by Lin and Liu (1990) that clinical signs such as abnormal movement and high respiration rate were induced by Tilapia (*Oreochromis mossambicus*) exposed to ammonia. The recovery period in this study was longer when compared with Ross *et. al*., (2002) who observed that rainbow trout exposed to 30-40 ppm rotenone gave good anaesthesia for 20-30 minutes. Histological examination showed varying degrees of degenerations in the lamellae, cellular infiltration, vacuolation, necrosis, pyknosis and alterations in the gills and liver (Table 3). Phenergan, like other toxicants, lead to physiological impairment in aquatic organisms (Warren, 1977; Aguigwo, 1998). The skin of *C. gariepinus* revealed severe damages. The skin lesions may have caused muscle dysfunction that led to stress and sluggish behavior of the fish.

Table 2. Behaviour of *C. gariepinus* exposed to different concentrations of phenergan.

Concentrations (ml)	0.0 ml	50 ml	100 ml	150 ml	200 ml	250 ml
Erratic Swimming	-	-	+	+	+	+
Opercula movements	-	-	+	+	+	+
Physiological stress	-	-	-	+	+	+

\+ indicates presence of particular observation
\- indicates absence of any physical observation

Table 3. Histological changes in gills and liver of *C. gariepinus* exposed to varying concentrations of phenergan.

Concentration(ml)	GILLS	LIVER
250	Complete degeneration of the gill filaments.	Thick appearance of the nucleus (pyknosis) and shrunken nucleus.
200	Cellular infiltration and some level of filament degeneration.	Thickening of the nuclear cell was observed.
150	There is high level of vacuolation and necrosis.	Serious cellular changes and tissues formation within the cells.
100	Low vacuolation, low degeneration and Slightly inflammation in cell tissues.	There is space formation in the tissue parenchyma.
50	Lamellae were intact and no vacuole was observed.	No serious cellular changes.
0 (Control)	Normal gill filament, normal lamellae.	Normal pathological features.

This study has shown that *C. gariepinus* exposed to phenergan produced similar anaesthetic effects as with other synthetic drugs in previous studies (Akinbulumo, 2004). Observations of abnormal rapid movements of the test organisms when treated with high concentrations of the phenergan solution suggested that the anaesthetic materials acted on the nerves of the catfish (Bowman and Rand, 1980). This study revealed that exposure to phenergan at 150ml to 250ml concentrations can produce significant changes in the physiology of *C. gariepinus* as manifested by the histological changes/damage, hence phenergan should be used at non sub-lethal levels of 50-100ml in aquaculture.

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