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Toxicity and Efficacy of Lidocaine as an Anesthetic for Nile Tilapia; *Oreochromis niloticus*

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Abstract: The anesthetic lidocaine was tested for its toxicity, efficacy and safety on Nile tilapia (*Oreochromis niloticus*). Toxicity values were variable and depended mainly on concentration and time of exposure. Concentrations of 60, 80 and 100 mg L^{-1} lidocaine were all effective for rapid anesthesia with 100% survival. In moderately rapid anesthesia, 40 mg L^{-1} lidocaine induced loss of equilibrium in Nile tilapia within 20 min exposure and 100% survival. Safety Index (SI) values for lidocaine on Nile tilapia indicated that the shorter the exposure time, the higher SI values. Repeated anesthesia in freshly prepared solution of lidocaine did not appear to affect on Nile tilapia sensitivity in terms of anesthetization, recovery and survival. However, results were in contrast and variable when previously prepared (old) solutions of lidocaine were used.

Key words: Nile tilapia, anesthesia, toxicity, efficacy

INTRODUCTION

Fish are easily stressed by handling and transport and stress can result in immuno-suppression, physical injury, or even death. In aquaculture anesthetics are required for a variety of fish management, culture, transportation, artificial spawning, tagging and experimental procedures. The main purpose of fish anesthetization is to reduce stress response and mortality. Worldwide, Quinaldine (2-methyl quinaline) and MS-222 (tricaine methane sulfonate) are the most commonly used fish anesthetics. However, quinaldine and MS- 222 have several disadvantages as they are expensive and sometimes not available in some countries, their safety indexes are low, their efficacies are affected by water chemistry characteristics, they are ineffective for long period of sedation and for quinaldine, fish retain some reflexes (Schoettger and Julin, 1967; Bell, 1987; Pirhonen and Schreck, 2002; Hedrick and Winmill, 2003; Palmer and Mensinger, 2004). Lidocaine is frequently used for its effect as a local anesthetic (Hunter-Griffin and Letha, 1991) and has been used in human and veterinary applications (Considine and Considine, 1984) and proposed to be suitable for fish anesthesia (Carrasco *et al*., 1984; Summerfelt and Lynwood, 1990; Salah El-Deen, 1998). Details of chemical pharmacology and toxicology for lidocaine are available in the physician's Desk Reference (Medical Economics Company, 1987; The Merck index, 1989; Ritchie and Greene, 1990).

In Egypt, Tilapias are one of the most important fish species for freshwater aquaculture and represent a major protein source in many of the developing countries. Although endemic to Africa, their distribution has been widened by artificial introductions, mainly since the 1950's to include much of the tropics and subtropics. Tilapias have many attributes that recommend them for culture. They show excellent growth rates on low protein diets, whether cropping natural aquatic production or receiving supplementary food. They tolerate wide ranges of environmental conditions, show little susceptibility to diseases and are amenable to handling and captivity. They have a short generation time and breed in captivity (Almazan and Boyd, 1978; Pullin, 1991; Abdel-Gawad *et al*., 2003; Agoz *et al*., 2005). Since anesthesia is used in different activities and quinaldine and MS-222 are not currently available, attentions have been focused to use alternatives that are inexpensive and

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available in the Egyptian market. The aim of the present study was to determine the toxicity and evaluate the anesthetic potential of lidocaine for use with Nile tilapia.

MATERIALS AND METHODS

Lidocaine (2%) solution was supplied by El-Nasr Pharm. Chemicals Company for El-Debeiky Pharma, Egypt, as lidocaine hydrochloride anhydrous. Nile tilapia fingerlings used in the present study were obtained from Abbassa Fish Farm ponds, 100 km north of Cairo, Egypt. The average total length of fish was 8.5±1 cm and average wet weight was 16.3±2 g. Fish were acclimated for two weeks in 2 m^3 fiberglass tanks before use. During the acclimation period, tanks were supplied with aerated and dechlorinated tap water. Fish were fed on a commercial pelleted diet (25% protein) every 24 h. Feeding was discontinued 48 h prior to and during the experiments. Fish were in good health condition and mortality was less than 1% during acclimatization. The water quality characteristics used in the present study were measured according to the methods described by APHA (1995) and are presented in Table 1.

Toxicity: Static toxicity tests were conducted in 80 L aquaria containing 70 L of water and to test fish. Required concentrations of lidocaine were stirred into aquaria that had caused 0-100% mortality in preliminary tests. There were triplicate aquaria for each concentration and for the control group (non-treated fish). All test aquaria were provided with a source of aeration till the end of the test. Fish responses to lidocaine were recorded to determine 5, 10, 20 and 40 min. LC_{50} values. Other groups were also tested daily to determine 24, 48, 72 and 96 h LC_{50} values (acute exposure). Dead fish were directly removed from the aquaria. Fish that remained anesthetized throughout the tests were placed in freshwater upon test termination. These fish were observed for two weeks after exposure in order to detect any delayed ill effects of lidocaine. Mortality data were analyzed by the method of and U.S.EPA (2002) and to determine lethal toxicity values (LC_{50}'s) and 95% Confidence Limits (CL).

Efficacy: Other groups of Nile tilapia were used to determine the efficacy of lidocaine by the method reported by Schoettger *et al.* (1967) and Limswan *et al.* (1983). Test conditions were the same as described for the toxicity tests. Behavioral responses of fish (Table 2) to lidocaine were used to determine five major stages of anesthesia and three major stages of recovery (Schoettger and Julin, 1967; Iwama *et al.*, 1989).

Efficacy was tested on 10 fish per replicate per concentration with a total of 30 fish. Usually one fish was exposed at a time and no more than two fish were anesthetized together. A concentration of Lidocaine was considered effective for rapid or moderately rapid when it induced stage 3b anesthesia within 5, 10 min or 20, 40 min, respectively and survival was 100%.

Safety index: Safety Index (SI) values were determined for 5, 10, 20 and 40 min exposures of Nile tilapia to lidocaine: $SI = LC_{50}/EC_{50}$; EC_{50} being the concentration that produced stage 3b anesthesia in half the specimens. The

Table 1: Physicochemical characteristics of test water

Characteristics	Mean	Characteristics	Mean
pH	7.2	Bicarbonate (HCO_3^-)	136.40 mg L^{-1}
Temperature	23°C	Carbonate (CO_3^-)	0.00 mg L^{-1}
Dissolved oxygen	6.7 mg L^{-1}	Sulfate (SO_4^-)	108.90 mg L^{-1}
Alkalinity	146.5 mg L^{-1} as $CaCO_3$	Chloride (Cl^-)	22.70 mg L^{-1}
Total Hardness (EDTA)	138.8 mg L^{-1} as $CaCO_3$	Calcium (Ca^{++})	37.60 mg L^{-1}
Ammonia (NH_3)	0.2 mg L^{-1}	Magnesium (Mg^{++})	8.80 mg L^{-1}
Ammonium (NH_4^+)	0.3 mg L^{-1}	Potassium (K^+)	4.90 mg L^{-1}
Nitrite (NO_2^-)	0.0 mg L^{-1}	Sodium (Na^+)	37.20 mg L^{-1}
Nitrate (NO_3^-)	1.6 mg L^{-1}	Electrical conductivity	0.50 m mohs cm^{-1}
Total soluble salts	332 mg L^{-1}		

Table 2: Stages of anesthesia and recovery (adapted from Schoettger and Julin, 1967; Iwama *et al.*, 1989)

Condition	Stage	Description
Anesthesia	1	Sedation, partial loss of reaction to external stimuli
	2	Partial loss of equilibrium, uncoordinated movement followed by active, erratic swimming
	3	Total loss of equilibrium
		a-Fish usually turns over but retains swimming ability
		b-Swimming ability stops but fish responds to pressure on the caudal peduncle
	4	Anesthesia, loss of reflex activity, fish fails to respond to external stimuli
	5	Modularly collapse, respiratory movement ceases (death)
Recovery	1	Body immobilized but opercula movements just starting
	2	Regular opercula movements and gross body movements beginning
	3	Equilibrium regained and preanesthetic appearance

Maximum Safety Indices (MSI's) were calculated from LC_1 and EC_{99} values obtained by the extrapolation of the regressions used in the determination of LC_{50} and EC_{50} values (Schoettger *et al*., 1967).

Repeated usage of old solution and repeated exposure: Groups of Nile tilapia (10 fish/trial with three replicates) which had not experienced anesthesia (unexposed fish) were subjected to be anesthetized ill previously prepared (24 and 48 h old) lidocaine solutions (60 mg L^{-1} determined from efficacy tests) for two successive times at 24 h intervals. Another group of Nile tilapia which was recovered from one time anesthetization was challenged to repeated anesthesia as mentioned earlier. A third group of Nile tilapia which had recovered from anesthesia was subjected to repeated anesthesia twice in freshly prepared lidocaine solution (60 mg L^{-1}) within a 24 h interval. In all conditions, time of anesthesia, recovery and survival (%) were recorded and the maximum exposure time used was 10 min (rapid anesthesia). In all conditions fish were directly transferred to freshwater for recovery as soon as signs of stage 3b anesthetization appeared otherwise they were left till the end of the experimental period.

RESULTS

Toxicity: The relevant-dose response data (LC_{50}'s and CL's) for lidocaine on Nile tilapia in terms of short term (5, 10, 20 and 40 min) and acute (24, 48, 72 and 96 h) exposures are presented in Table 3. In case of short term exposure, the 5 min LC_{50} with CL values of lidocaine was 127.40 (112.40-137.28) mg L^{-1}, whereas after 40 min exposure it was 50.79 (45.15-58.62) mg L^{-1}. In the acute exposure trials, the 24 h LC_{50} was 31.26 (14.31-58.28) mg L^{-1} lidocaine while after 96 h was 20.42 (6.40-65.12) mg L^{-1} lidocaine.

Efficacy: The efficacy of lidocaine for inducing rapid (5 and 10 min) and moderately rapid (20 and 40 min) anesthesia are presented in Table 4. Data were grouped without differentiation with those for each concentration and related exposure time. Anesthesia and recovery times as well as survival (%) varied with concentrations of lidocaine and induction time. Rapid anesthesia (stage 3b, at 5 and 10 min exposure) was determined using 40, 60, 80 and 100 mg L^{-1} lidocaine. No undue mortalities were experienced during experimentation. Anesthetization in 40 mg L^{-1} lidocaine exposure ranged from 2.11 to 9.55 min, while recovery time ranged from 40 sec to 10.3 min. In 60 mg L^{-1} exposure, ranges of anesthetization were from 1.0 to 5.57 min and recovery time ranged from 1.2 to 13.14 min. In 80 mg L^{-1} exposure, ranges of anesthetization were from 1.05 to 5.10 min and recovery time ranged from 20 sec to 4.0 min. While in 100 mg L^{-1} exposure ranges of anesthetization were from 50 sec to 4.20 min and recovery time ranged from 40 sec to 4.3 min. In case of moderately rapid anesthesia (stage 3b, at 20 and 40 min exposure), for Nile tilapia exposed to 40 mg L^{-1} lidocaine, the ranges of anesthetization time were from 1.30 to 11 min, recovery time was from 2.1 to 10.0 min and survival was 100% after 20 min and only 73% after 40 min exposure. In 60 mg L^{-1} exposure to lidocaine, Nile tilapia survival rate was 90% after 20 min and only 36% after 40 min exposure.

Safety Index (SI): In the present study, safety index values for Nile tilapia after exposure to lidocaine (Table 5) ranged from 2.54 to 3.24 and indicated that the shorter the exposure time, the higher the safety index.

Repeated exposure: The results of repeated exposure and repeated use of previously prepared (old) solution of lidocaine (60 mg L^{-1}) are presented in Table 6. Repeated anesthetization of the same fish in freshly prepared solutions did not appear to affect the sensitivity of Nile tilapia to lidocaine. However, some fish were slightly more tolerant than previously unexposed fish.

On the other hand, when previously prepared (old) solutions of lidocaine (60 mg L^{-1}) were used for anesthetization, the results were variable as some fish did not anesthetize and others anesthetized and recovered while the rest did not recover.

Table 3: Lethal Concentration (LC_{50}'s) with Confidence Limits (CL's) in parenthesis of lidocaine to Nile tilapia at different times of exposure

	Exposure time			
	5 min	10 min	20 min	40 min
LC_{50} mg L^{-1}	127.40 (112.40-137.28)	104.81 (95.83-110.94)	78.40 (69.30-89.39)	50.79 (45.15-58.62)
	Exposure time			
	24 h	48 h	72 h	96 h
LC_{50} mg L^{-1}	31.26 (14.31-58.28)	25.66 (17.65-49.77)	21.14 (4.70-75.12)	20.42 (6.40-65.12)

Table 4: Influence of lidocaine concentration (mg L^{-1}) and time of exposure (min) on rapid anesthesia status and survival of Nile tilapia. Fish were removed from test solution at the end of experimental period and transferred directly to freshwater for recovery

Conc. (mg L^{-1})	Exposure time	No. of Stage 3b	Time range of anesthesia (min) First	Time range of anesthesia (min) Last	No. not anesthesia	Recovery time range (min) First	Recovery time range (min) Last	Survival (%)
40	5	19	2.11	5.00	11	40.00 sec	02.00	100.0
	10	25	1.07	9.55	5	40.00 sec	10.30	100.0
	20	30	1.30	11.10	0	2.10	10.10	100.0
	40	30	1.35	11.40	0	4.05	10.10	73.0
60	5	25	1.00	5.00	5	1.20	11.59	100.0
	10	30	58.00 sec	5.57	0	3.00	13.14	100.0
	20	30	57.00 sec	7.30	0	2.30	11.55	90.0
	40	30	58.00 sec	6.25	0	7.21	13.01	36.0
80	5	26	1.05	4.50	4	20.00 sec	3.05	100.0
	10	30	1.10	5.10	0	2.10	4.00	100.0
	20	30	50.00 sec	5.30	0	6.30	11.00	76.7
	40	30	55.00 sec	6.00	0	8.33	13.20	13.3
100	5	29	50.00 sec	4.20	1	40.00 sec	8.31	100.0
	10	30	45.00 sec	4.44	0	4.10	18.30	90.0
	20	30	45.00 sec	3.40	0	9.00	14.27	40.0
	40	30	50.00 sec	3.25	0	Died	Died	0.0
120	5	30	20.00 sec	2.10	0	1.28	13.30	60.7
	10	30	18.00 sec	2.47	0	7.15	9.30	16.7
	20	30	22.00 sec	3.07	0	Died	Died	0.0
	40	30	15.00 sec	2.49	0	Died	Died	0.0

* Total of three replicates per concentration per time of exposure with a total of 30 fish

Table 5: Lethal Concentration (LC_{50}), Effective Concentrations (EC, stage 3b) and safety indexes of Nile tilapia exposed to lidocaine for rapid and moderately rapid anesthesia

Exposure time (min)	LC_{50} (mg L^{-1})	EC_{50} (mg L^{-1})	Safety index LC_{50}/EC_{50}	LC_1 (mg L^{-1})	EC_{99} (mg L^{-1})	Maximum safety index LC_1/EC_{99}
5	127.40	39.28	3.24	105.90	78.26	1.35
10	104.81	33.32	3.10	84.32	64.76	1.30
20	78.41	25.73	3.00	56.18	44.91	1.26
40	50.79	19.98	2.54	32.26	29.78	1.08

Table 6: Effects of repeated anesthesia of lidocaine (60 mg L^{-1}) and repeated use of previously prepared anesthesia solution on survival of Nile tilapia. Fish were removed from test solution when reached stage (3b) otherwise left till the end of experimental period (10 min). Anesthetized fish were directly transferred to freshwater for recovery

Conditions*	No. to Stage 3b	Time range of anesthesia (min) First	Time range of anesthesia (min) Last	No. not anesthesia	Recovery time range (min) First	Recovery time range (min) Last	Survival (%)
A	30	53.00 sec	5.57	-	3.00	13.14	100
B	19	2.50	5.20	11	4.25	10.35	85
C	12	4.50 sec	3.20	18	7.16	10.30	80
D	18	4.50 sec	3.20	12	3.10	5.50	70
E	11	3.10	5.25	19	4.25	9.50	60
F	30	2.00	8.10	-	3.02	60.00	100
G	30	2.50	9.10	-	6.35	10.20	100

Conditions* A: Freshly prepared lidocaine solution+unexposed fish, B: 24-old lidocaine solution+unexposed fish, C: 48-old lidocaine solution+unexposed fish, D: 24-old lidocaine solution+fish experienced anesthesia one time, E: 48-old lidocaine solution+fish experienced anesthesia two times, F: Freshly prepared lidocaine solution+fish experienced anesthesia one time, G: Freshly prepared lidocaine solution+fish experienced anesthesia two times

DISCUSSION

Toxicity: The obtained lethal concentrations of lidocaine demonstrated a definite and constant toxicity to Nile tilapia. Furthermore, the concentrations causing mortality can be predicted with a high degree of confidence (Table 3).

These toxicity trials gave the most variable results as shown by the relative width of the 95% confidence limits (Table 3). In several instances, during the experiment, the toxicity of 24 h exposure was nearly identical or higher with non significant differences compared to 48, 72 and 96 h LC_{50}'s. The reasons for such lesser variations in the LC_{50} values within the exposure times may include a decrease of drug concentration by absorption and metabolism in the fish, some natural degradation of the drug in solution by the time and the greater activity of the chemical on fish within the early hours of exposure (Marking, 1967; Palmer and Mensinger, 2004).

Also the relative width of confidence limit values may be attributed to a number of factors such as the individual variations of fish, their different susceptibilities to the drug and its metabolites and to the combined effect of fish excrement together with the drug and/or its metabolites

(Schoettger *et al*., 1967; Salah El-Deen, 1998; Hedrick and Winmill, 2003). In addition, although such variations in lethal concentrations either at short or acute exposure to lidocaine seem to be quantitatively different, but they are in each case, statistically not significant by difference from each other because there were overlaps in the confidence limits (APHA, 1995). Early works by Carrasco *et al.* (1984) revealed that LC_{50} values of lidocaine was 545 mg L^{-1} after 60 sec exposure to common carp (*Cyprinus carpio*), 492 mg L^{-1} after 90 sec exposure to catfish (*Ictalurus pinctatus*) and 2549 mg L^{-1} after 60 sec exposure to tilapia (*Oreochromis mossambicus*).

However, such results are comparatively different in magnitude from the present study in terms of many factors including species differences, fish size, water quality characteristics and induction time of the drug.

Efficacy: Such results reveled that although lidocaine could be used for rapid anesthesia at the lower concentration but there was a tendency of decreasing survival percentage with increasing time of exposure as well as concentration of the drug. Carrasco *et al.* (1984) reported that efficacy of lidocaine for common carp was 350 mg L^{-1} at 53 sec exposure period with 780 sec recovery time and for catfish was 250 mg L^{-1} at 88 sec exposure period with 754 sec recovery time. In the same experiment, the efficacy of lidocaine for tilapia was 250 mg L^{-1} at 179 sec exposure period and 422 sec recovery time and when the efficacy was 350 mg L^{-1}, the exposure period decreased to be 89 sec and recovery time increased to 612 sec. Such results are comparatively similar to the present study; however the relative difference may be attributed to species difference and water quality characteristics.

On the other hand, minor deviations in the efficacy of lidocaine were observed on Nile tilapia during experimentations. It is assumed that such variations may be partially related to the different susceptibilities of individuals and groups of fish and/or combinations of unknown factors (Summerfelt and Lynwood, 1990). Thus, it is highly recommended to conduct preliminary bioassays of anesthetic solution with several individuals from the fish stock which are to be narcotized (Schoettger *et al*., 1967; Summerfelt and Lynwood, 1990; Pirhonen and Schreck, 2002). Observations on the depth of anesthesia, exposure and recovery times can be used to determine the numbers of fish which can be anesthetized safely and minimize the risk of overexposures. Moreover, the cessation of opercular activity in fish during anesthesia may be considered a good criterion for transferring them to fresh water, although many individuals may recover completely from brief exposures beyond this point (Mattson and Riple, 1989; Molinero and Gonzalez, 1995). In addition, Tytler and Hawkins (1981) mentioned that, anesthesia may cause reduced gill ventilation due to depression of medullar respiratory centers, with hypoxia as a consequence. The hypoxia may be intensified by bradycardia and decreased blood flow through the gills. In the present study, at higher concentrations than 60 mg L^{-1} lidocaine, one might expect the development of anoxia and ensuing high mortality rates. The very short time required for recovery from lidocaine, however, alleviate the anoxia inflected. The short recovery time may be attributed to a rapid elimination of the drug during recovery, canceling the depression of the respiratory centers before oxygen depletion become critical (Anthony *et al*., 1996; Salah El-Deen, 1998). Recovery time can in some situations be critical. In the present study the time required for recovery from lidocaine was comparatively shorter than that required for other fish anesthetics (Mattson and Riple, 1989). This advantage together with a higher safety index should be emphasized when electing a suitable fish anesthetic.

Safety Index (SI): Shorter exposure may be desirable from the point of field and laboratory practices, but they may give less accurate results (Marking, 1967). Moreover, the obtained results in the present study showed that the Maximum Safety Index (MSI) is lower than SI and is biased in favor of greater safety (Table 5).

Similar findings on SI and MSI were also reported after anesthetization of rainbow trout, (*Onchorhynchus mykiss*) with MS-222 (Marking, 1967), channel catfish with etomidate (Limswan *et al*., 1983) and Chinook salmon, (*Onchorhynchus tshawytscha*) and Atlantic salmon (*Salmo salar*) with benzocaine (Gilderhus, 1990). The authors, however, advised to calculate both SI and MSI because one may suggest a possible hazard when the other does not.

Repeated exposure: The low number of fish which did not anesthetize and/or the decreased survival rate may indicate unfavorable effects of using old prepared lidocaine solution. However, one may suggest that the old solution may contain a lower concentration of lidocaine due to its degradation and/or the combined effect of lidocaine metabolites together with previously exposed fish metabolites in the solution. In addition the change in water quality may also affect on the anesthetization process. Such assumptions are highly supported by many investigations (Bell, 1987; Summerfelt and Lynwood, l990; Salah El-Deen, 1998).

Finally, in order to fully evaluate the characteristics of lidocaine as a fish anesthetic, further experimentations are necessary with regard to the drug elimination rate and efficacy at different water temperatures, water quality and with other fish species. It is also highly recommended to study the hematological, physiological and biochemical changes in fish exposed to lidocaine and compare results with other approved anesthetics such as MS-222.

From the obtained results we can concluded that 60, 80 and 100 mg lidocaine could be used in the rapid anesthesia (from 5-10 min) with a survival percentage 100%. And 40 mg lidocaine for moderately rapid anesthesia (20 min) with the same previous percentage of survival.

The safety index of the anesthesia on Nile tilapia increase with the decreasing of exposure time. Repeated a new prepared anesthesia for the same fish (3 times very 24 h), have no effect on recovery and survival rate comparing with the old prepared anesthesia.

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