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EVALUATION OF SOME ANAESTHETICS IN CATFISH (*CLARIAS GARIEPINUS*) ANAESTHESIA: A PRELIMINARY STUDY

(With 2 Tables)

By

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تقييم كفاءة بعض الأدوية المخدرة علي سمك القرموط: (دراسة مبدئية)

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أجريت هذه الدراسة لتقييم الكفاءة التخديرية لكل من الليدوكايين والكيتامين والزايلازين في سمك القرموط، وكان عدد الأسماك التي استخدمت في هذه الدراسة ٦٠ سمكة من كلا الجنسين ومتوسط وزنها ٣٥٤,٣٣جم ± ٩,٤جم (المدى ٢٩٠ــ ٥٠٥جم) جمعت من روافد نهر النيل بقنا. وقد قسمت ثلاثون منها إلى ستة مجموعات، استخدم الليدوكايين موضعياً بتركيز ٥ملل/لتر و١٠ مللي / لتر ماء في المجموعة الأولى والثانية ؛ كما استخدم كلاً من الكيتامين والزايلازين بالحقن العضلي وبجرعة ١٥مجم/كجم ٣٠مجم/كجم في المجموعة الثالثة والرابعة ؛ أما المجموعتين الخامسة والسادسة فقد تم الجمع بين الاستخدام الموضعي لليدوكايين بجرعة ٥ مللي / لتر والحقن العضلي لكل من الكيتامين والزايلازين بالجرعات المذكورة سابقاً. وقد تم قياس بعض المؤشرات البيوكيميائية في سيرم مجموعتين من السمك بعد ٢٤ ساعة من الإفاقة من التخدير بجرعتين مختلفتين من الليدوكايين (٥ مللي ، ١٠ مللي/ لتر) لكل مجموعة ومقارنتها بالمجموعة الضابطة. وقد أظهرت نتائج الدراسة بعد تحليلها إحصائياً ما يلي: ملائمة مستحضر الليدوكايين بتركيز ٥ مل/ لتر ماء للتخدير الموضعي في سمك القرموط حيث أعطت فقد كلي للاتزان وتخدير خفيف لمدة ١٤,٩ دقيقة ± ٢,٠٤دقيقة، وإفاقة تامة بعد ٧,١ دقيقة ± ٠,٣٥ دقيقة. زيادة الجرعة من الليدوكايين ≥١٠ مل/ لتر قد تؤدي إلى تأثيرات سمية وتغيرات في بعض المؤشرات البيوكيميائية. عدم ملائمة مستحضر الزايلازين لإحداث درجة مناسبة من التخدير إذا تم حقنه عضلياً في سمك القرموط. الجرعة المؤثرة من الكيتامين كانت ١٥ مجم/كجم و.ح. وقد أعطت درجات مناسبة من التخدير (نوم عميق: ٩ دقائق ± ٣,٨٩دقيقة ، نوم خفيف: ١٦,٥ دقيقة ± ٢,٢٣دقيقة) وإفاقة تامة بعد ٨,٧ دقيقة ± ٣,٥٦دقيقة. الجمع بين طريقتي التخدير الموضعي بالليدوكايين والحقن العضلي بالكيتامين أو الزايلازين في سمك القرموط قد يسرع من حدوث التخدير ويطيل من فترة الإفاقة (٦٧,٨ دقيقة ± ٧,٢٦دقيقة مع الكيتامين ،

١٤,٤ دقيقة ± ١٧,١دقيقة مع الزايلازين) مما قد يسمح بالتداول الروتيني في الاستزراع السمكي وإجراء بعض العمليات الأخرى.

SUMMARY

The Present work was conducted to evaluate lidocaine, ketamine, and xylazine as anaesthetics for catfish (*Clarias gariepinus*). A six groups of fish were subjected to definite concentration(s) of each anaesthetic rather than a combination of two anaesthetic agents (lidocaine/ketamine and lidocaine / xylazine). The external signs and behavior changes of the fish were observed and the time recorded per minutes during various stages of anaesthesia, recovery and survival in each group. The lowest effective concentration of lidocaine was 5 ml/l. when used as exposure anaesthetic, while that of ketamine was 15 mg/kg. b. wt. given intramuscular. Xylazine does not produce the desired stage of anaesthesia up to intramuscular injection of 30 mg/kg. b. wt. The induction of anaesthesia and recovery was found improved when lidocaine used in adjunct with ketamine or xylazine allowing delicate handling and invasive procedures to be performed. Serum samples obtained for determination of albumin, GOT, GPT, Creatinine, Urea, Cholestrol, Triglycerides, HDL and LDL Suggest that higher concentration of lidocaine $\geq$10ml/L.) may cause an adverse effects on tested parameters, and the survival rate 24 hours post exposure in catfish.

Key words: *Anaesthetics, catfish, clarias gariepinus*

INTRODUCTION

Anaesthesia is an invaluable and necessary technique for handling fish in order to submit them to procedures such as laparotomy, cannulation, hormonal induction and sanitary treatment (Jolly, Mawdesly–Thomas and Buche, 1972, cited in Brown, 1987 and Michael, 2002). In fisheries research and assessment, as well as in aquacultural operations, anaesthetics are essential for reducing stress and physical injuries during various handling procedures as sorting, weighing and measuring, tagging, sampling, spawning and transportation (Marking and Meyer, 1985; Gilderhus and Marking, 1987 and Cho and Heath, 2000). Also, certain anaesthetics as benzocaine may be useful against the monogenean skin parasite (*Entobedella hippoglossi*) in atlantic salmon (Svendsen and Haug, 1991). The effectiveness and safety of anaesthetics varies among fish species and depends upon water chemistry, other environmental factors and fish size (Schoettger and Julin, 1967 and 1969; Gilderhus and Marking, 1987).

Undesirable side effects have been reported for some anaethetics such as MS 222 which according to (Quinn, Olson and Konecki, 1988) harms the olfactory epithelium of catfish or causes tachycardia and hypoventilation, inducing a mixed respiratory and metabolic acidosis (Burleson and Smatresk, 1989). Benzocaine (ethyl–P–amino benzoate) is effective as a general anaesthetic for several species of fish but its residues was found to be deposited in most tissues of channel catfish (*Ictalurus punctatus*) up to 20 days after exposure to anaesthesia (William, Brain and Andrew, 1996). On the other hand, anaesthetics such as triacaine (MS 222) and quinaldine which have been widely used on fish are not always available in some countries. Injectable anaesthetics are suitable for large sized fish and when other techniques are inappropriate for use. Ketamine hydrochloride is an injectable, short–acting, dissociative anaesthetic agent and rarely used alone but rather in combination with other agents. A relatively high dose is required for immobilization and therefore is often used as an "induction" agent (Michael, 2002). Xylazine was used as injectable anaesthetic in sharks in a dose of 6 mg /kg b.wt (Harms, 1999). The best choice of an anaesthetic to be employed on to fish should combine the following characteristics: quick achievement of deep anaesthesia levels; ability to maintain the fish out of water for extended periods; reduced recovery times; possibility of repeated applications with no side–effects; low costs and availability (Martha and Alfonso, 1995). In the present study, we evaluated the effect of lidocaine, ketamine and xylazine when applied separately or in combination and determination of some possible adverse effects accompanying the use of lidocaine.

MATERIALS and METHODS

A- Fish:

A total of 60 catfish of both sexes with an average weight of 354.33 gm ± 9.4gm (range: 290-505gm) were catched from the Nile river tributaries at Qena city. Fish were apparently healthy and free from any skin lesions or external parasites. The fish were kept in glass aquaria supplied with well aerated and dechlorinated tape water for one week for acclimatization. The water was maintained at (22 ± 1 °C).

B- Anaesthetics:

- **Lidocaine Hcl** Debocaine 2% injection (20 mg /ml) produced by: El–Nasr pharm. Chemicals Co. for Al–Debeiky Pharma – A.R.E was used as surface anaesthetic in 5 ml and 10 ml / l. Alone or in combination with xylazine and ketamine in 5 ml/l. for each.

- **Ketamine Hcl** (Ketamar®) 50 mg / ml (product of Amoun Pharm. Co. S.A.E El-Obour city, Cairo, Egypt) was used in a dose of 15 mg/kg b.wt by IM injection.
- **Xylazine:** Xylaject injection 20 mg/ml. (Manufactured by: the Egyptian Co. for chemicals and pharmaceuticals (ADWIA) 10th of Ramadan City was used in a dose of 30 mg/Kg. b.wt by I/M injection. Intramuscular injection are given in the dorsolateral musculature dorsal to the lateral line using insulin syringe (1 ml). The needle should be inserted deeply and directed cranially to avoid retrograde drainage of the material from the injection site and also to minimize damage of the integument.

C- Experimental design:

Experiment (1): A total of 30 catfish were classified into 6 groups. The fish in group I and II were individually immersed in a glass aquaria containing lidocaine in a concentration of 5 ml/l. and 10 ml/l. The group III and 4 were subjected to ketamine 15 mg/Kg. b.wt IM and xylazine 30 mg/Kg b.wt. I.M respectively. A combined technique of administration was used in the last two groups as each fish exposed to lidocaine 5ml/L after intramuscular injection with ketamine 15 mg / kg b.wt (group V) and xylazine 30 mg/kg b.wt. (group V1). The efficacy of lidocaine, ketamine, xylazine and their combination as anaesthetics for catfish was evaluated using the guidelines of Kenneth, Michael, Ronald and Robert, (1995), and Miller (2000).

Experiment (2): This study was conducted to determine some adverse effects of lidocaine anaesthetic in 5 ml/l. and 10 ml/l. concentrations. A total of 30 catfish were classified into 3 groups: The control group received no anaesthetic, the second and third group were exposed to a dose of 5 ml/l. and 10 ml/l. lidocaine by immersion. Blood samples were collected from control and treated groups 24 hours post recovery via caudal vein. Clotted blood was centrifuged at 3000 r.p.m. for 15 minutes. Sera were separated and kept in refrigerator until used. Albumin was analyzed spectrophotometrically according to the method described by Doumas (1971), GOT was determined by method described by IFFC (1986), GPT activity was determined by king (1965), Urea was estimated by Searcy, Reardon and Forman, (1976), Creatinine was estimeted by the method of Houto (1985), Cholestrol was analysed by Schettler and Nussel (1975), Triglycerides were estimated according to Jacobs and VanDenmark, (1960), and finally HDL and LDL were estimated according to the methods described in National Cholesterol Education Program Recommendations (1995).

D- Statistical analysis: The results were recorded as the (mean ± S .E.) statistical significance was determined using analysis of variance according (Snedcor and Cochran, 1982). Means were compared by least significance Difference (LSD) test at 0.05 significance level. (Steal and Torrie, 1980).

RESULTS

No agent or combination of agents was considered 100% effective anaesthetic in all tested groups (Table 1). It was found that when concentration of lidocaine was increased (10 ml/l.) a significant decrease in the induction time as compared with the lower concentration (5 ml/l.) while the recovery was significantly more prolonged with the high dose tested group (10 ml/l.). Ketamine Hcl was found to induce a relatively light anaesthesia and a moderate recovery time in group III. In xylazine treated fish (group IV), the fish enters only to slight sedation after significantly long time, then the recovery was comparatively very rapid. When a concentration of 5ml/L. lidocaine Hcl was used in combination with ketamine Hcl 15 mg/Kg.b.wt) (growp V), the induction time was found significantly decreased while the recovery period was increased. At the meantime, when lidocaine Hcl 5ml/L. used in adjunct with xylazine (group VI), the fish passes through all the stages of anaesthesia with a little increase in induction and much more recovery time. In the present study, the fish was found to exhibit the behavioral and clinical signs of different stages of anaesthesia in all tested groups except in group IV (treated with xylazine only). Light sedation, the fish showed slow movement and slight loss of reaction to stimuli. The deep sedation, the fish showed slight decrease in opercular rate and muscle tone. Light narcosis, there were a weak response to postural changes, decreased muscle tone and increased excitatory phase and respiratory rate. Deep narcosis, there were a complete loss of equilibrium, decreased muscle tone and loss of reaction to stimuli. The light anaesthesia stage, the fish showed complete loss of muscle tone and decreased opercular and cardiac rate, respond to deep pressure and suitable for minor surgery (Table 1). The results in (Table 2), showed a significant increase in GOT, GPT and Albumin in lidocaine treated groups when compared with the control one. The creatinine values were not affected but a significant increase in urea was found when a dose of 10ml/L. lidocaine was used. Also, cholesterol, triglycerides and LDL were significantly increased at the same concentration, while HDL was found decreased in both treated groups.

Table 1: Stages of anaesthesia in catfish treated with lidocaine, xylazine, and ketamine Hcl.

Groups	Induction of Anaesthesia (min.)					Recovery from Anaesthesia (min.)		
	Light sedation	Deep sedation	Light narcosis	Deep narcosis	Light anaesthesia	Effort to change position	Response to stimuli	Normal stage
Group I lidocaine 5 ml/L.	2.6 ± 0.163^{cb}	4.3 ± 0.54^{b}	7.4 ± 1.35^{b}	10.7 ± 2.39^{a}	14.9 ± 2.04^{a}	4.2 ± 0.250^{d}	3 ± 0.333^{e}	7.1 ± 0.350^{cde}
Group II lidocaine 10 ml/L.	1.8 ± 0.133^{fe}	2.9 ± 0.46^{d}	5.2 ± 1.11^{cd}	6.2 ± 1.16^{cd}	6.8 ± 1.5^{bc}	7.7 ± 0.60^{c}	5.55 ± 0.77^{d}	21.8 ± 2.56^{b}
Group III ketamine 15 mg/kg b.wt by i.m.	2.8 ± 0.410^{b}	6.0 ± 0.615^{a}	16.5 ± 2.232^{a}	9.0 ± 3.899^{ab}	4.7 ± 1.955^{d}	3.8 ± 0.512^{de}	6.3 ± 0.920^{c}	8.7 ± 3.562^{cd}
Group IV xylazine 30 mg/kg b.wt by i.m.	15.3 ± 1.68^{a}	0.0 ± 0.0^{f}	0.0 ± 0.0^{f}	0.0 ± 0.0^{f}	0.0 ± 0.0^{f}	1.0 ± 0.0^{f}	1.0 ± 0.0^{f}	1.0 ± 0.0^{def}
Group V Lidocaine 5 ml/L + ketamine 15 mg/kg. b.wt by i.m.	2.6 ± 0.267^{bcd}	3.8 ± 0.533^{c}	5.2 ± 0.389^{c}	6.8 ± 0.442^{bc}	7.4 ± 0.499^{b}	18.4 ± 2.325^{a}	31.6 ± 0.957^{a}	67.8 ± 17.26^{a}
Group VI Lidocaine 5 ml/L + xylazine 30 mg/kg. b.wt by i.m.	2.2 ± 0.133^{bcde}	2.0 ± 0.0^{e}	2.8 ± 0.249^{e}	5.4 ± 1.108^{cde}	3.6 ± 0.163^{de}	10.0 ± 1.193^{b}	8.0 ± 1.174^{b}	14.4 ± 1.174^{bc}

a---- f : Means the values in the same column bearing different letters are significantly ($p < 0.05$) different, while those bearing the same letters are not significantly different.

Table 2: Some Biochemical parameters in catfish 24 hours post 5 ml/l. and 10 ml/l. of lidocaine treatments.

Parameters / Groups	GOT u/dl	GPT u/dl	Albumin g/ml	Creatinine mg/dl	Urea mg/dl	Lipid profile: Cholesterol mg/dl	Lipid profile: Triglycerides mg/dl	Lipid profile: HDL mg/dl	Lipid profile: LDL mg/dl
Control	68.80 ± 5.00 c	6.33 ± 0.463 c	0.730 ± 0.06 c	0.35 ± 0.03 a	9.03 ± 0.312 bc	141.53 ± 5.63 bc	103.0 ± 8.29 c	65.0 ± 4.11 a	55.93 ± 2.11 bc
5 ml/liter	76.30 ± 4.38 b	17.75 ± 0.479 b	1.23 ± 0.09 b	0.33 ± 0.03 a	9.21 ± 0.62 b	154.25 ± 4.11 b	208.0 ± 9.98 b	56.13 ± 2.94 bc	63.66 ± 3.36 b
10 ml/liter	86.80 ± [illegible] a	22.50 ± 1.06 a	1.44 ± 0.130 a	0.35 ± 0.05 a	10.88 ± 0.42 a	267.73 ± 16.57 a	238.89 ± 20.59 a	58.85 ± 4.52 b	167.84 ± 9.76 a

a,b and c : Means the values in the same column bearing different letters are significantly ($p < 0.05$) different, while those bearing the same letters are not significantly different.

DISCUSSION

The desired qualities for an anaesthetic are its ability to attain deep anaesthesia levels, keeping the organism at this state for periods which will allow their routine handling, along with reduced recovery times and no harmful side effects (Brown, 1993 and Martha and Alfonso, 1995). Taking into account the long evolutionary history of fishes and the many adaptations to different environment, no species can be used as a representative model for all fish (Catadi, Di Marco, Mandich, and Cataudella, (1998). Based upon the results illustrated in Table (1) and (2), it is clear that, none of the tested anaesthetic agent (s) is considered 100% effective. However, the present work points out the suitability of lidocaine as anaesthetic for catfish, as the fish show the similar classical behavior changes described in previously reported studies (Schoettger and Julin, 1976; Gilderhus and Marking, 1987; Olson *et al.,* 1995 and El-Nobi and El–Sharkawy, 2002). The time required for induction of anaesthesia was increased when the concentration of lidocaine increased with the increase of recovery time. This could be attributed to the cumulative effect of lidocaine. In the present study, the fish enters the first stage of anaesthesia after xylazine injection in a relatively long time (15.3 ±1.68) minutes and a very rapid recovery period (3 minutes). While, the anaesthetic dose in sharks is relatively very small, 6mg / Kg .b.wt, as reported by Harms (1999) as compared with that used in catfish. This could be due to species and environmental variation. Furthermore, the dose required to anaesthetize catfish is very high even when compared to that used in mammals. The fish exhibits the signs of each stage of anaesthesia with variable times with long periods of narcosis in comparison to other agents and a reasonable recovery time after ketamine injection. The dose used in this study is (15 mg / kg.b.wt) lies within the dose range detected in sharks, 12–20 mg/kg. b. wt., as described by Harms (1999). Another important point of preference for lidocaine is the ease of application as anaesthetic to fish if compared to intramuscular injection of xylazine and ketamine. The induction time in ketamine treated fish (group III) was near the values detected in (group I) (39.9–39.0 minutes for each) with a relatively much longer period of recovery (18.8–14.3 minutes). When a combination of lidocaine and ketamine was used in (group V), the induction and recovery values (25.8–117.8 minutes) are advantageously improved in comparison with the former values. This could be attributed to the synergistic action between the two agents. The same explanation could be mentioned for lidocaine /xylazine (group VI) as the fish enters all stages of anaesthesia with improvement in induction and recovery time (16 minutes and 32.4 minutes) advantageously compares with the values detected when each agent used separately. All of the aforementioned detected values in the present work were differ from that detected in other studies. Kenneth *et al.* (1995) defined the effective concentrations of anaesthetics for larval fish as those which induce stage 4 anaesthesia within 3 minutes after exposure with a recovery time of 10 minutes or less. The variation in values in the present work could be attributed to fish species difference, water chemistry, other environmental factors and fish size (Schoettger and Julin, 1967 and 1969 and Gilderhus and Marking, 1987). To elucidate the adverse effects of lidocaine with two different concentrations (5 and 10 ml / l.) on some biochemical parameters, blood serum will have to be checked and the values were compared with a control group fish. The significant increase in GOT, GPT, Albumin values in both treated groups suggest the anaesthetic stress effect on the fish liver. However, under the second concentration (10 ml/l.) mortalities were evident in 3 fishes after 24 hours (data not shown). A result which coincide with that reported on the effect of higher doses of tricaine methansulphonate (MS 222) as it causes 100 % mortalities at 0.4 mμ /L. (Nelson, 1953 and Houston and Words, 1972). In contrary, a prolonged high dose exposure of benzocaine does not induce any harmful effect in Nile tilapia (Allisson, *et al.,* 2003). The values of creatinine were not altered in both groups while a significant increase in urea concentration was observed in the higher dose treated group (10 ml/l.). This could be due to nephrotoxic effect of lidocaine. Likewise, it is clear that, the values of lipid profile were significantly altered after exposure of the fish to both concentrations to a various degrees which is prevalent at higher concentrations.

Based on the results of this study, the following conclusions can be made; (1) lidocaine is more effective and suitable for immersion anaesthesia in catfish; (2) ketamine is effective than xylazine Hcl as injectable anaesthesia in catfish; (3) The use of lidocaine as adjunct with ketamine or xylazine can improve the induction and recovery time in anaesthetized fish, thereby delicate handling and invasive painful procedures could be performed.

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