Study on anesthetic effects of Ketamine and Acepromazine in *Tor grypus*

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Abstract

Administration of human anesthesia drugs in fish is mainly because of their high efficacy and availability as well as low price. In this study anesthetic effects of Ketamine and Acepromazine (0, 15 and 30 mg L^{-1}) was evaluated in Shirbot (*Tor grypus*). Acepromazine concentrations prepared in 10 L containers in triplicates, then serial descending concentration of ketamine (for effective dose 20-400 mg L^{-1} and for lethal dose 320-900 mg L^{-1}) were added to tank. Twelve fish were added to each container. Results analyzed with probit software and EC_{10}, EC_{50} and EC_{90} as well as LC_{10}, LC_{50} and LC_{90} of drugs in five minutes were measured. Results showed that no significant difference was seen in anesthesia efficacy, time of anesthesia induction and recovery time among groups ($P>0.05$), but lethal effects of Ketamine decreased significantly in Ketamin along with 15 mg L^{-1} Acepromazine ($P<0.05$). Then it can be concluded that Acepromazine increased the safety of Ketamine in *Tor grypus* at low doses**.**

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Keywords: Ketamine, Acepromazine, *Tor grypus*, Anesthesia

Introduction

The *Tor grypus* (with common name shabbout or Shirbot) is one of the most important freshwater fish in Iran (Khuzestan, Lorestan, Kermanshah and surrounding provinces), Turkey, Iraq and Syria rivers (Nikpei 1996). Because of the ability to be alive in water with low oxygen and resistance to different levels of salinity and temperature this species of fish is suitable for cultivation (Baboli & Anvari 2018). Anesthetic drugs are several applications in aquaculture include handling, transportation and reducing trauma during invasive operations (Neiffer & Stamper 2009). Anesthetics are also used in fish during artificial spawning, weighing, tagging, grading, blood sampling, surgery and surgical procedures (Matin, Hossain & Hashim 2009; Anderson, McKinley

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& Colavecchia 1997). Anesthetics are used to lower the level of stress associated with such procedures. Overdose of anesthetics is also used routinely as an effective and humane means of euthanizing fish (Akbulut, Çakmak, Aksungur & Çavdar 2011). Many factors that impress choice of anesthetics are the convenience for use, safety for the fish, humans and the environment, effectiveness, physiological perturbations and the cost (Readman, Owen, Knowles & Murrell 2017). In the past, a number of different anesthetics have been used or evaluated for aquaculture applications such as quinaldine, benzocaine, 2-phenoxyethanol, etomidate,metomidate and tricaine methanesulfonate (Ross & Ross 2009). These anesthetics are always restrictions on consumption because of concerns about their health effects on humans or fish (Readman et al. 2017). The most commonly used fish anaesthetic is tricaine methanesulfonate (MS-222). Tricaine is expensive and requires a 21-day withdrawal period before fish can be consumed (Popovic, Strunjak-Perovic, Coz-Rakovac, Barisic, Jadan, Persin Berakovic & Sauerborn Klobucar 2012). Also, Ketamine is a general anesthetic drug used in medicine and veterinary science (Lorrain, Baccei, Bristow, Anderson & Varney 2003). Ketamine (Kalipsol, Ketalar, Ketanest and etc) is a fast-acting anestheticdrug for half a century which has other clinical applications such as analgesia, sedation, neuroprotective, anti-inflammatory, sympathetic nervous system stimulation and antitumor effects (Molina, Moyano, Serrano-Rodriguez, Ayala, Lora, Serrano-Caballero 2015). Ketamine alone has a species-specific effect in fish often characterized by incomplete anesthesia, respiratory disturbance, and prolonged recovery with excitement. For this reason to reduce dosage-related side effects and to improve anesthesia safety and depth of anesthesia ketamine is often combined with another drug (Kawai, Takagi, Kaneko & Kurosawa 2011; Ross & Boss 2009). An ideal anesthetic for fish should induce anesthesia in less than 5 min and be safe to users as well as being inexpensive and easy to use. Furthermore, anesthetic should not have permanent and persistent effects on the behaviour and physiology of the fish (Neiffer & Stamper 2009). Acepromazine (ACP) is the most commonly used phenothiazine (PHE) for sedation and administered to reduce excitement and stress during various veterinary procedures, and as a pre-anesthetic agent (Nishimura, Villela, Carvalho, Borges, Silva & Mattos-Junior 2017). Its sedative effect is a consequence of antagonism of D2-dopaminergic receptors in the central nervous system, histamine-1 and 5-HT2 serotonergic receptors (Vesal, Sarchahi, Nikahval & Karampour 2011). Because of lack reports on the use of ketamine-Acepromazine combinations in fish, we examined the anesthetic effect, lethal dose, time of anaesthesia induction and recovery time of ketamine in combination with Acepromazine in *Tor grypus.*

Materials and Methods

Four hundred sixty-two juveniles *T. grypus* with 4.2 ± .45 g mean weight of either sex were

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obtained from Hamidieh fish farming of Ahvaz. One week prior to start the experiment, fishes were randomly distributed in three 300 L fibreglass tanks to adapt to a new situation. The fish were fed 3 percent body weight daily and two days before the experiment was off fed.

Water quality factors were recorded during the experiment as: temperature, 25 ± 1°C; Dissolved oxygen, 8-10 ppm; pH, 7.9 ± 0.3; NO_2, <0.01 ppm and NH_3, <0.1ppm. The water exchange rate was 10% of water volume. A preliminary pilot study was performed to achieve proper dosage for induction a light anaesthesia stage in fish so that low respiratory rate, total loss of equilibrium, no effort to right itself, decrease muscle tone, reactivity to strong tactile and vibratory stimuli (Ross & Boss 2008). According to preliminary pilot study, three experimental groups of fish were established (Seven dose in triplicates), G1: fish anesthetized with different concentration of ketamine without acepromazine, G2: fish anesthetized with combination of 15 mg L^{-1} acepromazine and serial concentration of ketamine, G3: fish anesthetized with combination of 30 mg L^{-1} acepromazine and serial concentration of ketamine. One group was maintained without anesthesia as a control (Table 1).

Table 1. Different concentration of acepromazine and ketamine to determine an effective dose of ketamine-Acepromazine combinations

Groups	Concentrations of Acepromazine (mg L^{-1})	Number of treatments	Concentrations of Ketamine (mg L^{-1})
1	0	7	0, 20, 40, 80, 160, 320, 400 (triplicates)
2	15	7	0, 20, 40, 80, 160, 320, 400 (triplicates)
3	30	7	0, 20, 40, 80, 160, 320, 400 (triplicates)

Twelve fishes were added to each 10 L tank. Time to anesthesia and recovery from anesthesia of each fish were recorded for each treatment, anesthesia was confirmed by observing cessation of voluntary swimming and loss of responses to pinching the skin with forceps and pricking the tail (Al-Hamdani, Ebrahim & Mohammad 2010). To allow the fish to recover from anesthesia, the fish smoothly were transferred into fresh, untreated dechlorinated water in 10-l tanks. For determining the lethal dose of ketamine-Acepromazine combination, the same method was designed but, the higher dose of ketamine was used (Table 2). Fishes were exposed to each anaesthesia dose for 5 minutes, afterwards the fish were transferred to -anesthetic-free water. After an hour, the fish was considered dead which stayed immobile in the bottom of the tank and opercula movement had stopped. To determine the effective dose (EC) and lethal dose (LC) the data was analyzed by probit (version 1.5) software.

Table 2. Different concentration of acepromazine and ketamine to determine lethal concentration

Group	Concentrations of Acepromazine (mg L^{-1})	Number of treatments	Concentrations of Ketamine (mg L^{-1})
1	0	7	0, 320, 400, 560, 620, 800, 920 (triplicates)
2	15	7	0, 320, 400, 560, 620, 800, 920 (triplicates)
3	30	7	0, 320, 400, 560, 620, 800, 920 (triplicates)

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Results

The results of effective concentration for EC_{10}, EC_{50} and EC_{90} of ketamin and Acepromazinewere (Mean ± SD) showed in table 3 and figure 1.

Table 3. Effective concentrations of acepromazine and ketamine in experimental treatments

Treatments	Acepromazine concentrations (mgL^{-1})	Ketamine concentrations ($mg\ L^{-1}$)	EC_{10}	EC_{50}	EC_{90}
1	0	0, 20, 40, 80, 160, 320, 400	32.3 ± 6.4	58.8 ± 6.4	86.0 ± 3.5
2	15	0, 20, 40, 80, 160, 320, 400	28.0 ± 12.4	50.5 ± 5.7	82.7 ± 13.6
3	30	0, 20, 40, 80, 160, 320, 400	31.0 ± 3.5	52.8 ± 3.2	84.3 ± 0.6

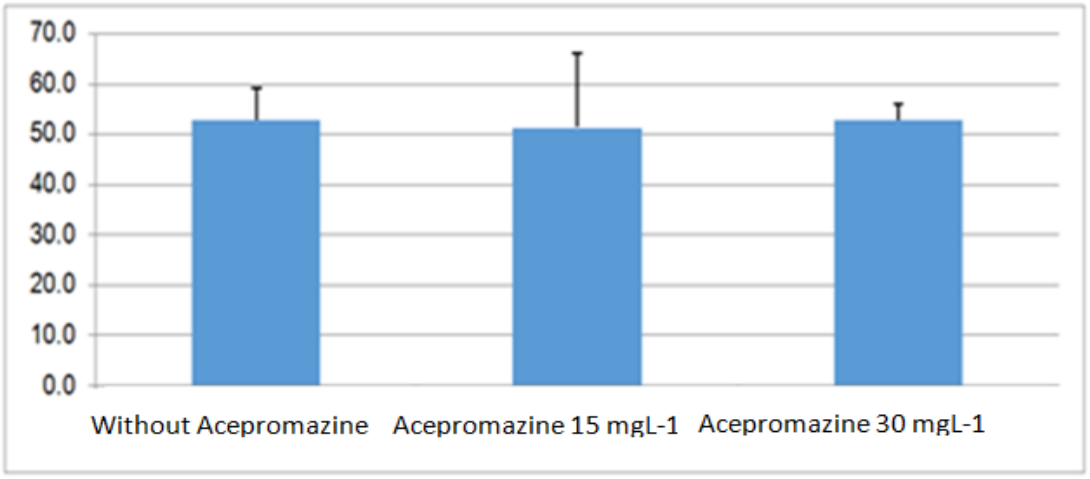

Figure 1. Comparison of EC_{50} at (Mean ± SD) different concentrations of Ketamine and Acepromazine.

The results of effective concentration for EC_{10}, EC_{50} and EC_{90} of ketamin and Acepromazinewere showed in table 3 and Figure 1. The results showed that different concentrations of acepromazine had a minor effect on EC_{50} levels of ketamine, and these changes were not significant statistically. Based on the results of tables 3 and 4 and probit analysis effective and lethal doses of ketamine were 40 and 320 mg L^{-1} respectively. According to the results of Table 4, the use of 15 mg L^{-1} acepromazine reduced the toxic effects and LC_{50} of ketamine ($P<0.05$) but in the higher dose (30 mg L^{-1} acepromazine) had no effect on reducing toxicity and LC_{50} (Figure 2).

Table 4. Lethal Concentrations of Acepromazine and Ketamine in Experimental Treatments

Treatments	Acepromazine Concentrations ($mg\ L^{-1}$)	Ketamine Concentrations ($mg\ L^{-1}$)	LC_{10}	LC_{50}	LC_{90}
1	0	0, 20, 40, 80, 160, 320, 400	378.3 ± 8.3	600.5 ± 26.7	755.3 ± 66.9
2	15	0, 20, 40, 80, 160, 320, 400	608.3 ± 24.0	730.2 ± 22.3	876.5 ± 23.8
3	30	0, 20, 40, 80, 160, 320, 400	258.0 ± 13.9	439.7 ± 19.6	747.3 ± 39.6

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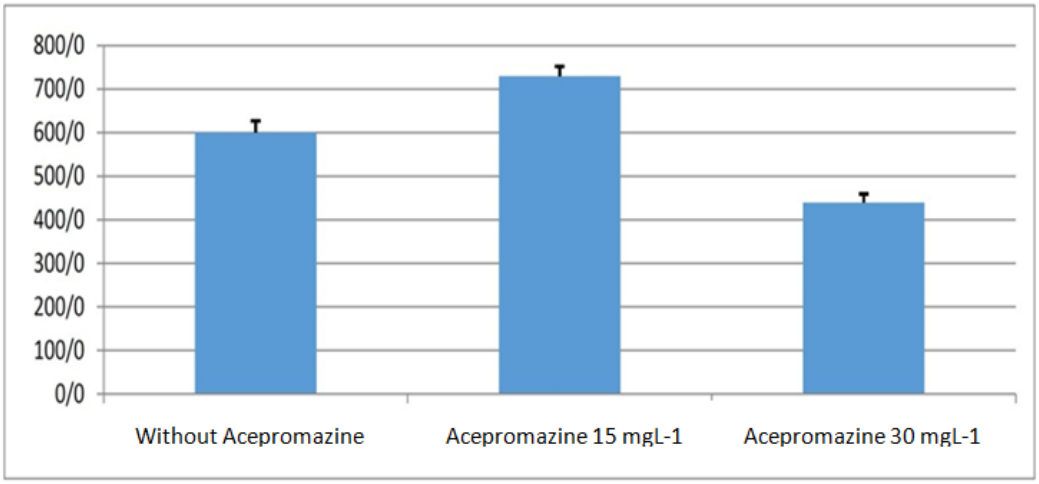

Figure 2. Comparison of LC_{50} (Mean ± SD) in different concentrations of Ketamine and acepromazine.

Since acepromazine reduced ketamine toxicity, mortality pattern (Table 5) showed that the use of 15 mg L^{-1} of acepromazine significantly decreased mortality in Different concentrations of ketamine lethal doses.

Table 5. Mortality rate (mean ± SD) in three treatments of acepromazine with different concentrations of ketamine.

Acepromazine (30 mg L^{-1})	Acepromazine (15 mg L^{-1})	Acepromazine (0 mg L^{-1})	Ketamine concentration (mg L^{-1})
4 ± 0	0 ± 0	0 ± 0	320
4 ± 2	0 ± 0	0 ± 0	400
6 ± 0	0 ± 1.1	4.6 ± 1.1	560
9.2 ± 1.1	2.6 ± 0	8 ± 0	620
11.2 ± 1.1	7.2 ± 1.1	12 ± 1.1	800
12 ± 0	12 ± 0	12 ± 0	920

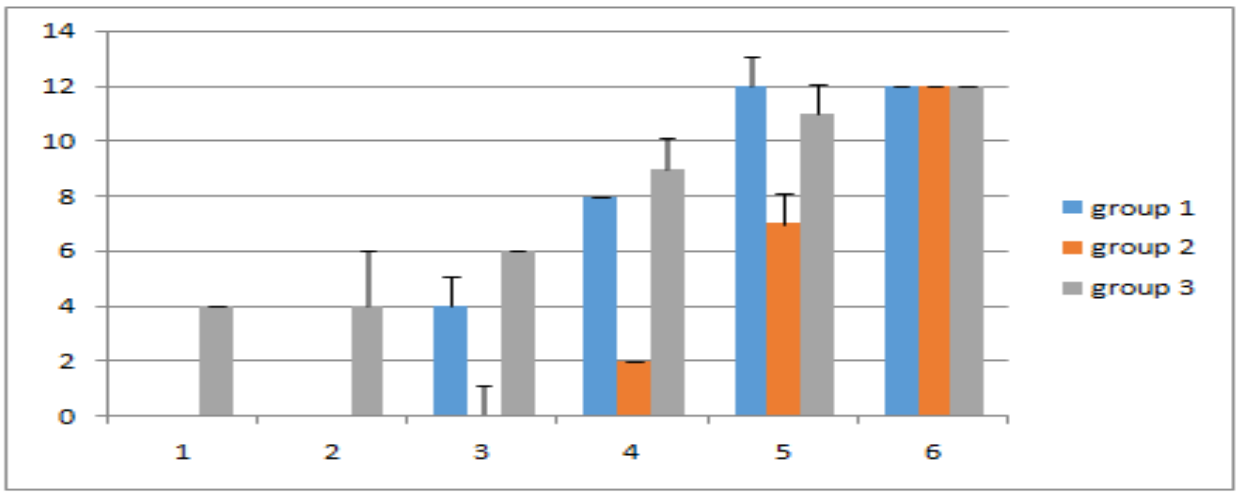

Figure 3. Mortality rate (Mean ± SE) in the three treatments (Different concentrations of acepromazine and ketamine).

According to the results, it is concluded that the same dose of acepromazine (15 mg L^{-1}) significantly reduced the ketamine anesthetia induction time ($P<0.05$), but no change was observed in the recovery time ($P>0.05$).

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Discussion

Although an anesthetic drug, the tricycain methane sulfonate (MS 222), is highly preferred to other anesthetics such as ketamine (Mirzargar, Soltani, Ahmadi, Abrishamifar, Bahonar & Yousefi 2011). But due to the high cost and limited access to it, an alternative anesthetic drug has been studied in various studies, for example, Clove oil (Velisek, Svobodova, Piackova, Groch & Nepejchalova 2005) and Phenoxy ethanol (Hedayati 2018). Limited studies have been conducted on the use of ketamine as an anesthetic drug in fish (Peyghan, Baniadam & Amirdad. 2001). The combination of anesthetic drugs reduces their toxic effects (Aboeldahab et al. 2011). In this study, acepromazine was used to reduce the toxic effects of ketamine. Ketamine apparently has a species-specific effect often characterized by incomplete anesthesia, respiratory disturbance, prolonged induction and excitement recovery when used alone as an anesthetics in fish (Al-Hamdani et al. 2010; Riehl, Kyzar, Allain, Green, Hook, Monnig & DiLeo 2011). Combination of other drugs with ketamine increased anesthesia efficacy and reduced side effects of kethamin in various researches (Martins, Diniz, Félix & Antunes 2018). Combination of Anesthetics with other drugs usually used to improve safety, efficiency, the depth of anesthesia and reduce the need for high doses of anesthetics. (Heidari Peyghan, Esmaeilir-Rad, Najafzadehvarzi, Bita & Poormehdi 2014). In this study acepromazine (15 mg L^{-1}) with ketamine in Compared to ketamine significantly reduced ketamine toxicity ($P < 0.05$) and anesthetic induction time. However, no effect was observed on the dose of induction anesthesia ($P > 0.05$). It should be considered higher dose of acepromazine (30 mg L^{-1}) did not have such a beneficial effect on ketamine toxicity. Comparison of anesthesia Induction with ketamine in *Tor grypus* with other fishes showed this species is more resistant to anesthetic effects of ketamine. For example, EC_{50} and LD_{50} of ketamine in common carp at 15 minutes was 35 and 170 mg L^{-1} respectively. However, the combination of ketamine with xylazine (15 mg L^{-1}) changed EC_{50} and LD_{50} to 36.3 and 230 mg L^{-1} respectively (Peyghan et al. 2001). Concurrent use of ketamine and xylazine caused increased the anesthesia induction and recovery time in common carp (Al-Hamdani et al. 2010). There are several investigations that combined various drugs (particularly sedatives) in order to increase ketamine efficacy. Fleming et al, showed that combination of medetomidine and ketamine despite increasing of ketamine efficacy, also decreased its side effects including bradycardia and apparent respiratory depression in *Acipenser oxyrinchus* (Fleming, Heard, Floyd & Riggs 2003). Besides Marco et al, indicated that combined medetomidine and ketamine affected physiological parameters (blood hypercapnia, respiratory acidosis and stress response) to a lesser extent in comparison to ketamine and MS222 in sturgeon hybrid (Marco, Petochi, Longobardi, Priori, Finoia, Donadelli & Marino 2011). These results

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indicated that the combination of medetomidine with ketaimine is in agreement with our results. Apparently, both medetomidine and acepromazine have synergistic effects with ketamine to suppress CNS and magnify the anesthetic effects of ketamine. In contrast Al-Hamdani et al. (2010) reported that combination of xylazine with ketamine increased time of anesthesia induction, but the duration of anaesthesia was longer which is sufficient for common clinical and surgical interventions. The difference between metabolic variations and species differences are important factors that contribute to differences in responses of fish to anesthetic agents (Zahl, Kiessling, Samuelsen & Hansen 2009). Smith and Bastos-Ramos indicated xylazine and fentanyl increased anesthesia efficacy of ketamine in grey nurse sharks and Antarctic fish (Smith 1992; Bastos-Ramos, Goncalves & Bacila 1998). The Results of this study also indicated that 15 mg L^{-1} of acepromazine reduced ketamine toxicity to *Tor grypus*. Although lethal concentration (LC_{50}) of acepromazine (15 mg L^{-1})-ketamine were significantly higher than ketamine ($P<0.05$) but the amount of 30 mgL^{-1} of acepromazine decreased LC_{50} of ketamine (Higher toxicity). It seems that acepromazine in low concentration decreased side effects of ketamine, but a higher concentration of acepromazine increased adverse effects of ketamine in *T. grypus*. Toxic effects of ketamine were reported in various fish species. Riehl et al. (2011) reported similar results of toxic effects of ketamine on serum biochemical parameters and mortality of zebrafish (Riehl *et al* 2011).

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Conflict of interests

The authors declare that there is no conflict of interest.

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مطالعه اثر بیهوشی کتامین و آسه پرومازین در ماهی شیربت *Tor grypus*

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چکیده

تجویز داروهای بیهوشی انسانی در ماهی به دلیل در دسترس بودن و قیمت پایین و کارایی بالا انجام می‌شود. در این مطالعه اثر کتامین و آسه پرومازین (صفر، ۱۵ و ۳۰ میلی‌گرم در لیتر) در ماهی شیربت (*Tor grypus*) ارزیابی گردید. غلظت‌های آسه پرومازین در مخازن ۱۰ لیتری در سه تکرار آماده شده و غلظت‌های کاهشی کتامین (دوز مؤثره ۲۰- ۴۰۰ میلی‌گرم در لیتر و غلظت کشنده ۳۲۰-۹۰۰ میلی‌گرم در لیتر) به مخازن اضافه گردید. ۱۲ ماهی به هر مخزن اضافه شده و بیهوشی ماهی‌ها در هر مخزن ثبت گردید. نتایج با نرم افزار پروبیت آنالیز گردیده و غلظت مؤثره EC_{10} ، EC_{50} و EC_{90} همچنین غلظت کشنده LC_{10}، LC_{50} و LC_{90} در محدوده زمانی ۵ دقیقه مشخص گردید. نتایج نشان داد که تفاوت معنی‌داری بین کارایی بیهوشی، زمان القای بیهوشی و زمان بازگشت از بیهوشی بین تیمارها مشاهده نگردید ($P>0.05$)، ولی غلظت کشنده کتامین با تجویز ۱۵ میلی‌گرم در لیتر آسه پرومازین بطور معنی‌داری کاهش نشان داد ($P<0.05$). لذا می‌توان نتیجه گرفت که آسه پرومازین در افزایش محدوده ایمنی کتامین در ماهی شیربت مؤثر است.

کلمات کلیدی: کتامین، آسه پرومازین، ماهی شیربت، بیهوشی

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