A comparison between sedation using propofol and clove oil in Levantine scraper (*Capoeta damascina*)

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Abstract
Anesthetic agents are used in fish to decrease stress and mortality during sorting, surgery, diagnosis and artificial fertilization procedure. This study was done to evaluate and compare the efficacy of propofol and clove oil for anesthetizing *Capoeta damascina*. For this purpose, 40 fish were caught from Arvand River and divided into two groups. After a pilot study, propofol and clove oil were examined in 12.5 mg L⁻¹ and 125 mg L⁻¹ concentrations, respectively. The onset of anesthesia, duration period of anesthesia and recovery time were recorded separately for each group and compared. Results showed that the anesthesia onset of specimens in propofol treatment, was significantly shorter than that of clove oil treatment (*p*<0.05). Also, recovery time in propofol treatment was longer (*p*<0.05) than that of clove oil. Based on the results, it was recommended to use propofol instead of clove oil in performing anesthesia for this species due to rapid induction, longer duration and more prolonged effect in immersion method.

Keywords: Clove oil, Propofol, Anesthesia, Immersion method, *Capoeta damascina*

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Introduction

Anesthetic and analgesic drugs are mainly used in aquaculture industry to reduce the stress and prevent mortality in fish during transportation, handling, artificial insemination, examination, biopsy, manipulation, surgical procedures, sorting and marking (Serezli et al., 2012; Bahrekaizemi and Yousefi, 2017). The most commonly anesthetics used in aquaculture industry are Tricaine methanesulfonate (MS222), 2-phenoxyethanol, benzocaine, methomidate, etomidate, ketamine hydrochloride, lidocaine, diazepam (Stoskopf, 1993), aqueous and alcoholic extract of clove oil (Velisek et al., 2005), Nicotiana tabacum (Zargham et al., 2013) and Zataria multiflora Boiss (Sharif Rohani et al., 2008).

Propofol (2, 6-diisopropyl-phenol) is an ultra-short–acting sedative agent, with a molecular weight of 178 Da and pH between 7- 8.5 (Hall et al., 2001). Previous studies have characterized the dose response with propofol for a number of reptiles such as green iguanas (Knotkova et al., 2005) and also, for a number of fish species including Acipenser oxyrinchus, De soti (Fleming et al., 2003) spotted bamboo sharks (Chiloscyllium plagiosum), (Miller et al., 2005), Benni (Barbus sharpeyi), (Mortazevi Zadeh et al., 2012) and gold fish (Carassius auratus). (Gholipour Kanani and Ahadizadeh, 2013).

Clove oil is among the most important agents used for fish sedation in the reproduction and restocking centers. The main constituents of clove oil are eugenol (80-90 %), eugenol acetate (15%) beta caryophyllene (5-12%), and also, gallotannic acid, caryophyllene oxide, α-humul and calcium oxalate (Taylor and Roberts, 1999). Among the benefits of clove oil are low price, availability, safety and minimal side effects to fish. There are a number of studies on the clinical use of clove oil as an anesthetic in several fish species (Davis and Griffin, 2004; Bagheri and Imanpour, 2011; Mohammadi Arani et al., 2013).

Levantine scraper, with scientific name of Capoeta damascina, is one of 28 species of the genus Capoeta which found throughout the Middle East, especially in Iran (Gorshkova et al., 2002). This fish lives in different part of Iran especially in Arvand River, Daryacheh-ye Maharlu, Hormuz and Hamun-e Jaz Murian basins (Ghorbani Chafi, 2000; Esmaeili et al., 2010) and is important for recreational fishing in rivers. Unfortunately, few studies conducted considering anesthesia on this valuable species. The main objective of the present study was to compare the effects of propofol and clove oil (as the two most commonly used anesthetics) for anesthetizing C. damascina and to determine the appropriate dose for these anesthetic agents.

Materials and methods

Totally 40 samples (Levantine scraper) were collected by Salic net (a cast net)
from Arvand River of the Karoon branch, in Chaharmahal-va-Bakhtiari Province. Samples were kept in plastic bags which were filled with 1/3 water and 2/3 oxygen and transferred to veterinary surgery, University of Shahrekord. Standard length and weight of all samples were measured before anesthetic test.

The average fish sizes used in the experiments were 98 ±10 g in weight and 210.6± 18.2 mm in length. Fish samples were adapted to the new condition while kept in glass aquaria containing river water for one week. Dissolved oxygen, pH, water temperature, salinity and total hardness were continuously measured throughout the experiments with average values of 6.4±0.63 mg L⁻¹, 7.8±0.23, 21.5±1.34°C, 3.4±0.22 ppt and 254.2±6.5 mg L⁻¹ respectively. Fish feeding was stopped 24 hours before the beginning of the experiments.

Propofol 10% (Rapinovet ®) obtained from Caspian Rasht Company and clove oil from Barij Esans Company. At initial step, a pilot study was conducted with concentrations of 2.5, 5, 7.5, 10 and 12.5 mg L⁻¹ of propofol and 0.25, 0.5, 0.75, 1.0 and 1.25 g L⁻¹ of clove oil. Based on the obtained results, 12.5 mg L⁻¹ of Propofol and 125 mg L⁻¹ of clove oil was selected as appropriate dose for next step.

Samples were divided into two groups (n=20) with three replicates and exposed to specific concentrations. The onset of anesthesia, the time taken to reach total loss of equilibrium (at stage 3 with symptom including; total loss of reaction to even massive stimulation) and recovery time were recorded. As soon as exposed fish achieved the stage 3 of anesthetizing, they were immediately transferred to other glass aquaria equipped with aerated water to determine the recovery time. The data were subjected to t- test statistical analysis (p<0.05) using SPSS version 18.

**Results**

According to RP-HPLC, major components of clove oil were eugenol (82%), eugenol acetate (11%) and beta caryophyllene (4%). No mortality was observed utilizing the concentration determined in the pilot study performed to determine the minimum effective dose. The onset of the anesthesia, duration period of anesthesia and the recovery time caused by propofol and clove oil in *C. damascina*, expressed in seconds (s) are shown in Table 1. In particular, the anesthetic propofol activity (23.2±1.744) in the onset of anesthesia was significantly (p<0.05) higher than that of clove oil (40.80±14.2), the anesthesia time caused by propofol (42.8±4.727) was significantly (p<0.05) shorter than that of clove oil (80.2±18.49) and the recovery time following propofol anesthetization (623±8532) was significantly (p<0.05) longer than that following clove oil treatment (467.2±91.92).
Table 1: The onset of anesthesia, duration period of anesthezia and recovery time (Mean±SD) caused by propofol and clove oil in Capoeta damascina (n= 20).

<table>
<thead>
<tr>
<th></th>
<th>Propofol</th>
<th>Clove oil</th>
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<tbody>
<tr>
<td>The onset of anesthesia (S)</td>
<td>23.2±1.744a</td>
<td>40.80±14.25b</td>
</tr>
<tr>
<td>Duration period of Anesthesia (S)</td>
<td>42.8±4.727a</td>
<td>80.2±18.49b</td>
</tr>
<tr>
<td>Recovery time (S)</td>
<td>603±28.87</td>
<td>467.2±91.92</td>
</tr>
</tbody>
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Different alphabetic superscripts in the same row indicate significant differences between compared groups (p<0.05).

Discussion
Propofol is a selected anesthetic agent in veterinary medicine, because of the rapid induction and metabolism, low side effects, rapid recovery, non-toxic effect in different organs and negligible influence on liver metabolism (Short and Bufalari, 1999). It is showed that the phenolic derivative of propofol has a role in the strong sedation and weak analgesia propofol (Kay and Stephenson, 1980). Results of the current study showed that induction time with propofol was significantly shorter than that of clove oil (p<0.05). Also, recovery time with this drug was longer compared to clove oil (p>0.05). Similar to our results induction time caused by propofol in Gulf of Mexico sturgeon (Acipenser oxyrinchus) was significantly shorter than Medetomidine-Ketamine (Gregory et al., 2003). In Mortazevi Zadeh et al. (2012) study, the concentration of 4-6 mg/L of propofol had the optimum performance in total anesthesia and recovery time of B. sharpeyi. According to the pilot doses we used 12.5 mg/L propofol as a proper dose while Peyghan et al. (2008) reported an optimum dose of propofol in grass carp (Ctenopharyngodon idella) anesthesia for immersion and injection 4 mg/L and 2 mg/kg, respectively. In Gholipour Kanani and Ahadizadeh (2013) study, induction time by propofol in C. auratus was significantly longer than that of Clove oil (p<0.05), although, in recovery time no significant difference was observed between the two studied drugs.

Clove oil is a common anesthetic agent in aquaculture, in addition to anesthetic effects and the rapid recovery time, few side effects and lack of environmental impacts were associated with intravenous and inhalation anesthetics (Taylor and Roberts, 1999; Mohammadi Arani et al., 2013). By dissolving the clove powder in water, it is absorbed through the gills and cause paralysis in central nervous system which is attributed to clove ingredients (eugenol). This paralysis starts from the cerebral cortex and progress to spinal cord and finally affects the vital centers of the medulla oblongata and causes restless swimming followed by an erratic swimming and then immobilization and fall into a state of unconsciousness in the back or side (Bagheri and Imanpour, 2011).
In different studies, the effects of clove oil as anesthetics agent is demonstrated on several fish species (Hall et al., 2001; Velisek et al., 2005; Gomulka et al., 2015). The duration time of anesthesia with essential oil of clove in 125-25 mg/L concentrations in the Persian sturgeon was less than 10 minutes (Mohammadi Arani et al., 2013), in the rainbow trout fingerlings for breeders at concentrations of 50, 100, 150 and 200 ppm were between 1 to 5 minutes (Iwama et al., 1989) and in three groups of Persian sturgeon fingerlings between 4-13 minutes (Mohammadi Arani, 2006). However, in this study the duration time of anesthesia in C. damascina was between 7-8 minutes.

Reliable, fast and light induction, appropriate anesthesia for surgical interventions and minimal suppression of vital organs can be noted as good features of propofol as anesthetic agent (Peyghan et al., 2008). In addition, in different studied fish species rapid recovery, continuous and complete anesthesia was reported (Miller et al., 2005; Mortazevi Zadeh et al., 2012; Gholipour Kanani and Ahadizadeh, 2013).

In conclusion, the use of propofol in immersion method compared to clove oil showed rapid induction of anesthesia which lasted longer and more constant. So, propofol appears to be more efficacient anesthetic for C. damascina than clove oil. However, in this study only clinical effects of propofol and clove oil were examined on C. damascina. More investigations are needed to compare the effects of propofol and clove oil on liver enzymes activity, respiratory and heart rate, physiological response and changing in hematolgy values of C. damascina.

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