



Anesthetic Activity of Clove Oil (Eugenol) on the Lake Van Fish (*Chalcalburnus tarichi* Pallas, 1811)

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ABSTRACT The use of clove oil (eugenol) as an anesthetic for Lake Van fish (*Chalcalburnus tarichi*), an endemic cyprinid fish species of Lake Van basin, was examined. Induction times were determined to be ≥ 35 , < 4 , < 3 , < 2 , < 2 and ≥ 1 min at 20, 40, 50, 60, 80 and 100 mg/L clove oil concentrations, respectively. Recovery times upon 10 min of exposure to eugenol were determined respectively to be > 3 , > 4 , > 3 , > 4 , > 6 and > 8 min clove oil can be considered as a suitable anesthetic for *Chalcalburnus tarichi* and an optimum anesthetic concentration of 50 mg/L is recommended.

Keywords: Anesthesia, *Chalcalburnus tarichi*, Eugenol

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Van Gölü İnci Kefali (*Chalcalburnus tarichi* Pallas, 1811) Balığında Karanfil Yağının (Öjenol) Anestezik Etkisi

Karanfil yağının Van Gölü Havzası endemik balığı olan inci kefalinde anestezik olarak kullanılması incelenmiştir. 20, 40, 50, 60, 80, 100 mg/L konsantrasyonlarında indüksiyon zamanları sırası ile ≥ 35 , < 4 , < 3 , < 2 , ≥ 1 dk olarak tespit edilmiştir. Balıkların öjenole 10 dakika maruziyetten sonra uyanma süreleri sırası ile > 3 , > 4 , > 3 , > 4 , > 6 , > 8 dk olarak belirlenmiştir. Öjenol *Chalcalburnus tarichi*'de de uygun bir anestezik olarak kabul edilebilir ve optimum anestezik konsantrasyon 50 mg/L olarak önerilmektedir.

Anahtar Kelimeler: Anestezi, *Chalcalburnus tarichi*, Öjenol

INTRODUCTION

Anesthetics play important roles in fishery research and culture as they are used in weighing, height measurement, intake of eggs and sperm, marking and prevention of animal stress in various veterinary practices, which are all carried out manually (Çetinkaya and Şahin 2005; Hajek et al. 2006). During those manual practices, such common fish anesthetics astringine (MS222), quinaldine sulfate, benzocaine and phenoxy ethanol are commonly used. However, anesthetics may cause certain side effects, including gill irritation, corneal damage and general poisoning in fish. Therefore, clove oil can be used as an alternative anesthetic because of its economic benefits and nontoxic characteristics. Being a natural product with active component of eugenol, clove oil is thus recommended as an effective and inexpensive fish anesthetic (Inoue et al. 2003; Hajek et al. 2006). This fish species lives in Lake Van basin and is of considerable local economic importance. It is consumed as fresh and salted by the people living around the Lake Van basin (Duyar 2000).

The aim of this study was to investigate the anesthetic activity of clove oil on the Lake Van fish (*Chalcalburnus tarichi*).

MATERIALS and METHODS

Chalcalburnus tarichi were caught with throw-net from their habitat of Karasu stream that flows into the Lake Van and brought to the test field in aerated containers. The fish with 8.7 ± 1.25 cm were used in the experiments. Upon placing in a fiberglass tank of 800 L filled with dechlorinated tap water with ventilation the fish were fed with commercial trout pellets for 7 days of acclimation. During the acclimation period and experiments, water temperature was maintained at 20.4 ± 0.35 °C, pH at 8.39 ± 0.17 , dissolved oxygen at 6.7 ± 0.8 , dissolved oxygen saturation at $79\% \pm 10$, electrical conductivity at 839.9 ± 10.37 µS/cm, total hardness as CaCO₃ at 328 ± 14 mg/L, and total alkalinity as CaCO₃ at 514 ± 8 mg/L (Anonymous 1995).

The experiments were carried out in a glass aquarium containing 60 L of water. Ten fish for each group were exposed to eugenol for 10 min eugenol was diluted 1:10 in 95% ethanol because of its water insolubility and a 100 mg of stock working solution for anesthesia experiments was prepared. The duplicate experiments were performed in 7 groups at eugenol concentrations of 20, 40, 50, 60, 80 and 100 mg/L (Inoue et al. 2003; Hajek et al. 2006) and 1:10 diluted 95% ethanol solution (Ünsal 1998; OECD 2000; EPA 2002a; EPA 2002b; Çetinkaya 2005).

Data were statistically analyzed by analysis of variance (ANOVA) using SPSS 11.5 for Windows. Different means were compared using Duncan's multiple-range post hoc test. The results were expressed as the mean \pm standard error of the mean and the differences were considered statistically significant at $p < 0.01$. Research Approval Certificate for the study was granted by the Local Ethic Committee of Animal Experiments at Van Yuzuncu Yil University (Decision Number: 2011/01/09).

Table 1. Anesthesia induction times on *Chalcalburnus tarichi* (n=10)

Clove oil (mg/L)	Induction time (seconds) ¹	Wake-up time (seconds)
20	2100.0 \pm 94.3 ^d	202.0 \pm 77.6 ^a
40	224.4 \pm 81.1 ^c	268.0 \pm 43.1 ^a
50	174.2 \pm 74.3 ^{bc}	230.0 \pm 44.5 ^a
60	104.2 \pm 12.9 ^{ab}	283.0 \pm 44.5 ^a
80	103.6 \pm 20.8 ^{ab}	378.0 \pm 76.5 ^a
100	65.0 \pm 14.8 ^a	514.0 \pm 60.7 ^a
Control	0.0 \pm 0.0	0.0 \pm 0.0

Values are expressed means \pm standard error. Same letters in the same column indicate similar values ($p < 0.01$).

DISCUSSION

Inoue et al. (2003) reported that the clove oil anesthetic effect at 40 mg/L on *Bryconcephalus* juveniles taken place in 1 min and that the wake up from anesthesia occurred independent of the concentration. Hajek et al. (2006) studied anesthetic effects of clove oil on *Cyprinus carpio* and reported that 30-50 mg/L concentration was safe and very effective. In a study aiming at the determination of acute toxicity and anesthetic effects of clove oil on *Penaeus semisulcatus* under various water quality conditions, Soltani et al. (2004) reported that when clove oil concentration was increased, the time required for sedation and anesthesia was shorter. The results of this study were in agreement with the results of other researchers and proved that eugenol in various concentrations can be used as an anesthetic agent on *C. tarichi*. Although the results shown in Table 1 focused on 50 and 60 mg/L concentrations, as the amount of given anesthesia increases on *C. tarichi*, the wakeup time is surprisingly delayed with lengthened time for entering induction. As seen in Table 1, clove oil concentration about 50 mg/L was enough to anesthetize the fish in <3 minute and the wake up time was independent in regard to anesthetic concentration. Therefore, optimum anesthetic concentration is 50 mg/L for *C. tarichi*.

CONCLUSION

As a result, eugenol, the extract of clove oil used commonly in dentistry for many years, appears to be an alternative anesthesia in fisheries. This study proves that eugenol is an acceptable anesthetic for *C. tarichi*.

RESULTS

Table 1 includes the induction and wake up times of *C. tarichi* subjected to various eugenol concentrations.

Induction times were determined to be ≥ 35 , <4, <3, <2, < 2 and ≥ 1 min at 20, 40, 50, 60, 80 and 100 mg/L clove oil concentrations, respectively. Recovery times upon 10 min of exposure to eugenol were determined respectively to be >3, >4, >3, >4, >6 and >8min.

There were significant differences between the groups ($p < 0.01$). The longest induction time was attained at 20 mg/L eugenol concentration. There were no statistically significant difference in induction times at 60, 80, 100 mg/L concentrations. These three concentrations also get into the same period of time in induction. The anesthetic effect was not observed in the control group.

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