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Evaluation of the Anesthetic Effects of Cove Oil (*Syzygium aromaticum*) on Lorestan Newt (*Neurergus kaiseri*)

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ABSTRACT

The present study describes the anesthetic effects of clove oil (*Syzygium aromaticum*) in Lorestan newt (*Neurergus kaiseri*). 6 adult newts (n = 3 for each group) with 13 ± 2 gr were used. The newts were initially divided into two groups, with three newts per group. Each group was subjected to one of two final concentrations [0.3 and 0.5 ml/l] of clove oil. The sedation, anesthesia, and recovery duration were recorded based on the behavioral events after exposing the newts to each aquarium. After inducing anesthesia, the newts were transferred to anesthetic-free aquariums, and recovery duration was recorded. The study showed that anesthesia was achieved in newts across two different concentrations using clove oil. Sedation occurred faster with a higher concentration (0.5 ml/l), taking 7.33 ± 1.52 minutes, compared to 10.66 ± 1.15 minutes with the lower concentration (0.3 ml/l). However, anesthesia lasted longer with the higher dose, averaging 22.66 ± 3.05 minutes, versus 15 ± 2.64 minutes for the lower dose. Recovery was also slower with the higher concentration, indicating stronger anesthetic effects with increased doses of clove oil. Our study supports clove oil as an effective anesthetic in *Neurergus kaiseri* and recommends its application for achieving suitable induction, anesthetic duration, and recovery profiles.

Introduction

Lorestan newt (*Neurergus kaiseri)*, a species endemic to Iran, is among the three species of Neurergus located in the southern Zagros Mountains, specifically within the provinces of Lorestan and Khuzestan. This species is easily recognizable by its distinctive orange stripe and white spots. Classified as critically endangered, the survival of newt is at risk due to its decreasing population and limited habitat in the area. Furthermore, threats to its habitat and diseases such as red foot syndrome significantly endanger its existence.[1,2]

The decline in amphibian populations worldwide has increased the scientific community's interest in studying these creatures in natural and controlled environments. Handling amphibians can cause stress and requires general anesthesia for various procedures, such as clinical interventions and biological sampling. It is essential to understand how different anesthetic substances affect these animals to ensure the success of these procedures. Additionally, these substances can also be used as euthanasia agents to end the lives of amphibians in field studies humanely or to manage specific stages in the life cycle of invasive amphibian species when used in high doses.[3,4]

Amphibians can be anesthetized using various methods, including injection, inhalation, topical application, or immersion in an anesthetic solution.

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There is increasing interest in veterinary practice regarding amphibians' medical treatment and surgical procedures. Historically, frogs were commonly anesthetized using tricaine methanesulfonate (MS-222).[5] The US Food and Drug Administration regards it as a potentially hazardous substance. Research has demonstrated that direct exposure to MS-222 can damage the retina in amphibians, fish, and humans.[6]

Various agents have been used as anesthetics in amphibians through different methods of administration, including subcutaneous, intramuscular, intracoelomic, and intravenous routes. For example, systemic injections of ketamine[7] and other cyclohexanones, such as the combination of tiletamine with zolazepam,[8] volatile anesthetics like methoxyflurane and isoflurane given topically or via a water bath,[9-11] intravenous injections of propofol and intracoelomic injections of medetomidine or barbiturates have been employed.[7,12,13] The diverse anesthetic medications resulted in inconsistent levels and durations of anesthesia, which varied within and across species, between genders, based on animal weight, and with different administration methods.[13]

Clove oil, primarily composed of eugenol, is recognized for its use in dental care for humans due to its local anesthetic and antiseptic properties and as a cost-effective and readily available alternative for anesthetizing amphibians. Its effectiveness in pain relief and anesthesia in amphibians adds to its appeal.[14]

This study aims to investigate the effect of clove oil on the anesthesia of NG for monitoring and interventional purposes.

Materials and Methods

The code of ethics LU approved the present experimental study. ECRA.2024.88 in Lorestan University.

The essential oil of cloves was obtained from the flowers and stems of the clove by hydro-distillation with a Clevenger-type apparatus. GC-MS analyzed the clove oil to determine its components (Table 1). Six adult newts with 13 ± 2 gr weight were housed in a pre-prepared and oxygenated aquarium (50 L) for one week to equalize the environmental conditions (25 °C, pH = 7.5) (Figure 1). An extra aquarium was also considered for the recovery of the anesthetized newts. A semi-static system was used, and 50% of the water volume was changed daily. The newts fasted 24 hours before the experiments.

The depth of anesthesia in amphibians is evaluated by considering several reflexes, including the escape response, righting reflex, pain response, and palpebral reflex. The escape response involves observing whether the animal tries to flee when held in an open palm. However, not all animals may exhibit this behavior due to their stoic or captive nature. The righting reflex is tested by flipping the amphibian onto its back and timing how long it takes to flip itself upright again. Sedated animals show slower responses and anesthetized animals do not manage to right themselves. The palpebral reflex is checked by lightly touching the eyelid to see if the animal blinks, indicating alertness. The response to superficial pain is measured by pinching the skin, typically at the rear feet. The disappearance of the superficial pain reflex occurs before the loss of the deep pain reflex, which is the first to return during recovery.[15] The study outlines three phases related to anesthesia: sedation, the anesthesia itself, and recovery. Sedation is marked by the cessation of voluntary movement and a partial loss of balance. The anesthesia phase is when the animal no longer responds to intense external stimuli, including significant tactile sensations, and lacks a righting reflex. Recovery is characterized by the gradual return of responses to both superficial and deep pain, the restoration of the righting reflex, and the animal beginning to move again.

The experiment involved using the smallest number of animals possible, which aligns with the policy of reducing the use of experimental animals. The newts were divided into two equal groups (n = 3). They were immersed entirely in the prepared solution and the whole surface of the newt's body was in contact with the solution. The newts in the first group were exposed to water with clove oil at 0.3 ml/l. The newts in the second group were exposed to water with clove oil at a 0.5 ml/l concentration. Our dosages were similar to those of other amphibians.[16] All groups were individually placed in containers containing the determined concentration of these plant extracts.

Three observers recorded the time of induction times for each stage separately to minimize experimental error. Solutions with clove oil concentrations of 0.3 ml/l and 0.5 ml/l in the water were prepared, and each group of newts was exposed to one of these concentrations. They were immersed entirely in the prepared solution. The times were noted and then compared. Once the anesthesia was induced, the subjects were moved to the recovery aquariums. After the end of the experiment, all the newts were safely returned to their natural habitat.

All results were analyzed using SPSS version 26. Sedation, anesthesia, and recovery duration data were collected and analyzed using a paired t-test. All the measurements were expressed as mean ± SD, and differences were considered significant at $p < 0.05$.

Results

According to the results in Figure 2, sedation was produced in all newts in two groups with clove oil in different concentrations. The sedation duration in 0.5 ml/l concentrations was significantly shorter than in 0.3 ml/l concentrations ($p < 0/05$). For a group with 0.3 ml/l

concentration of clove oil, the average time for sedation duration was 10.66 ± 1.15 min, and for a group with 0.5 ml/l concentration, the average time for sedation duration was 7.33 ± 1.52 min.

Anesthesia duration was significantly longer in the high-dose group ($p < 0.05$). The average anesthesia duration for the low-dose group was 15±2.64 min, and for the high-dose group was 22.66±3.05 min. The newts were limp in this duration.

Recovery duration in 0.5 ml/l concentrations was more prolonged than 0.3 ml/l but this difference was insignificant ($p < 0.05$). In comparison, the anesthetic effects of the high-dose clove oil are roughly more than the low-dose group.

Discussion

Comparing the anatomy and physiology of amphibians and mammals presents distinct challenges and benefits concerning anesthesia. Amphibians possess a unique ability for transcutaneous gas exchange, attributed to their significant surface area-to-volume ratio and a minimally thick epidermis overlaying a well-vascularized dermis. This trait facilitates the rapid absorption of pharmacological agents via their skin, enabling the application of immersion and topical anesthesia techniques. However, the literature on amphibian anesthesia and analgesia is scarce, with a limited number of recommended pharmaceuticals. Clove oil emerges as an economical and productive anesthetic among the viable options. The primary component of clove oil, eugenol, exhibits pharmacodynamic properties akin to established anesthetics such as benzocaine and MS-222 by hindering neural signal transmission, altering membrane functionality, and directly influencing cerebral and spinal cord activities.[17-20]

Our findings reveal that the average time required for induction of anesthesia in the low-dose and high-dose groups was 10.66 ± 1.15 minutes and 7.33 ± 1.52 minutes, respectively. Following this period, all subjects, specifically salamanders, achieved a level of anesthesia sufficient for easy handling, evidenced by the loss of the righting response. The salamanders remained limp for an average duration of 15 ± 2.64 minutes and 22.66 ± 3.05 minutes, respectively, after being transferred from the container with clove oil solution to one containing water. The findings of our study corroborate existing literature on the subject of amphibian anesthesia. Notably, a study investigating the anesthetic efficacy of clove oil on a large-sized salamander (*Amphiuma tridactylum*) demonstrated that exposure to clove oil for 5 minutes was adequate for conducting minimally invasive procedures. For

Table 1. GC.MS analysis for clove oil. The main components of clove oil are eugenol, trans-caryophyllene, aceteugenol, and beta-selinene.

Compound	Area%
2-Heptanone	0.296477
4-Octanol,7-methyl-, acetate	0.371803
2-Nonanone	0.25315
Linalool	0.108223
Benzyl acetate	0.211216
Methyl salicylate	0.807748
Trans-anethole	0.203586
Eugenol	82.42526
Trans-caryophyllene	7.966507
Beta-selinene	1.220431
Aceteugenol	4.63382
Caryophyllene oxide	0.398625

Figure 1. Morphology of *Neurergus kaiseri*.

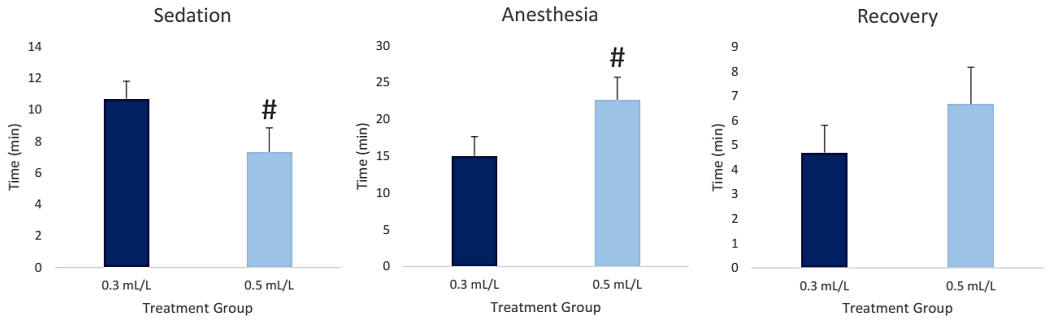

Figure 2. Influence of different concentrations of clove oil in three stages of anesthesia in *Neurergus kaiseri*. (# indicates a significant difference between two groups with $p < 0.05$).

procedures demanding a higher degree of invasiveness, such as intraperitoneal surgery, it was determined that a minimum exposure of 10 minutes was necessary. Recovery time has a reverse ratio to salamanders' weight. With increased weight, decreased recovery time.[16] This insight adds a valuable dimension to our understanding of anesthetic recovery dynamics in amphibians, particularly about their physical attributes.

Comparatively, a study on tiger salamander anesthesia indicated a quicker induction with a clove oil bath than with an intracoelomic injection of propofol, attributing this efficiency to the drug's rapid integumentary absorption. The median anesthesia induction time was 12 minutes.[14]

Furthermore, research examining the influence of eugenol on anesthetizing frogs revealed limited negative consequences within 24 hours. Moreover, pharmacokinetic evaluations indicated a half-life of four hours, suggesting the potential for accumulative impacts following consecutive administrations. This highlights the importance of exercising caution regarding frequent application.[21]

In conclusion, our study supports clove oil as an effective anesthetic in *Neurergus kaiseri* and recommends its application for achieving suitable induction, anesthetic duration, and recovery profiles. This review underscores the need for further research into amphibian-specific anesthesia to refine and expand the available methodologies and agents, thereby enhancing the welfare of these animals in research and clinical settings.

Conflict of Interest

There is no conflict of interest to declare.

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