Iran Agricultural Research (2025) 44 (1) 131-142.

Shiraz University

Iran Agricultural Research

Journal homepage: https://iar.shirazu.ac.ir

Research Article

Evaluation of anesthetic potency of peppermint extract (*Mentha piperita*) and its physiological and histological effects on the Persian sturgeon (*Acipenser persicus*) juveniles

Sara Vali*, Mohammad Mazandarani, Mohammad Sudagar, Mohammad Reza Imanpour

Department of Fisheries, Faculty of Fisheries and Environmental Sciences, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, I. R. Iran

ARTICLE INFO

Keywords:
Anesthesia
Freshwater fish
Herbal extract
Toxicity

ABSTRACT- Anesthesia is a practical method for reducing stress in aquatic organisms. This study assessed the effectiveness of peppermint extract as an herbal anesthetic, along with its physiological and histopathological effects on Persian sturgeon (*Acipenser persicus*). Ninety fish were exposed to peppermint extract at concentrations of 0, 150, 250, 500, 750, and 1000 mg/L. To evaluate the effects of the solvent, an additional 75 fish were exposed to ethanol at concentrations of 450, 750, 1500, 2250, and 3000 mg/L. Ethanol exposure did not produce significant anesthesia, physiological changes, or tissue damage ($P > 0.05$). Peppermint extract successfully induced general anesthesia, particularly at concentrations between 300 and 500 mg/L, with both induction and recovery times averaging under five minutes. However, concentrations above 500 mg/L led to hematological changes and moderate tissue damage. Observed lesions included gill hyperplasia, congestion, epithelial lifting, liver degeneration and vacuolization, and necrosis in kidney tubules. Hematological analysis showed significant increases in hematocrit and hemoglobin levels, indicating possible stress or respiratory impairment, while red blood cell and white blood cell counts remained unchanged compared to the controls. A concentration of 150 mg/L was effective in reducing stress, while 300–500 mg/L is recommended for achieving optimal anesthesia.

INTRODUCTION

Anesthesia is defined as a temporary loss of sensation or awareness (Bodura et al. 2018), and it can occur at different levels depending on its application (Chance et al., 2018). General anesthesia, on the other hand, suppresses central nervous system activity, leading to unconsciousness and a complete loss of sensation. It represents the deepest and most controlled form of reversible anesthesia, typically used to prevent movement, muscle contraction, and pain during handling or procedures (Gomulka et al., 2008). In aquaculture, common challenges such as high stocking densities, environmental pollution, transportation stress, and disease outbreaks can significantly affect fish health and productivity (Vajargah et al., 2019., Vajargah et al., 2021). Anesthesia has proven to be an effective tool for mitigating stress and managing fish behavior during various routine and invasive procedures, including reproduction, transportation, sorting, sampling, vaccination, and surgery (Faggio et al., 2014; Sattari et al., 2020). A variety of chemical anesthetics, such as tricaine methanesulfonate (MS-222), quinaldine sulfate, and phenoxyethanol, are widely used for inducing relief and general anesthesia in fish (Elumalaia et al., 2020; Faggio et al., 2014). However, their use is associated with several limitations. Prolonged exposure to MS-222, for instance, has been shown to significantly elevate stress-related blood parameters such as cortisol, glucose, lactate, and hematocrit in fish (Boijink et al., 2017), which are recognized indicators of physiological stress (Coyle et al., 2004). Additionally, phenoxyethanol has been reported to cause skin lesions and blisters in fish (Stoskopf and Posner, 2008). Beyond their direct effects on aquatic organisms, chemical anesthetics raise concerns regarding food safety and environmental persistence (Ortuno et al., 2002). As a result, natural anesthetic alternatives have gained increasing attention. Compounds like eugenol have demonstrated effective anesthetic properties in multiple fish species. For example, Tarkhani et al. (2017) evaluated eugenol across different size classes of angelfish (*Pterophyllum scalare*), confirming its suitability for aquaculture applications. Herbal anesthetic extracts offer several advantages: they are cost-effective, widely accessible, easy to administer, and typically exhibit low toxicity to aquatic animals (Ortuno et al., 2002; Holloway et al., 2004; Evans et al., 2005). Moreover, these plant-derived compounds are generally safe for human consumption and environmentally degradable (Evans et al., 2005). In recent years, several herbal extracts, such as clove,

* Corresponding Author: Department of Fisheries, Faculty of Fisheries and Environmental Sciences, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, I. R. Iran
E-mail address: saravaali1989@gmail.com
https://doi.org/10.22099/iar.2025.52182.1664
Received 29 January 2025; Received in revised form 26 April 2025; Accepted 29 April 2025
Available online 8 June 2025

lemon beebrush, Avishan-e-Shirazi, and peppermint, have been explored for their anesthetic properties in aquaculture, often showing superior performance compared to conventional chemical agents (Imanpoor et al., 2010; Gaikowski et al., 2001). For instance, Hoseini and Ghelichpour (2012) demonstrated that clove extract effectively induced anesthesia in Beluga (*Huso huso*) without significantly altering hematological parameters. Similarly, Yousefi et al. (2018) compared the anesthetic efficacy of citronellal and linalool in juvenile common carp (*Cyprinus carpio*), finding that citronellal, a major component of Cymbopogon distillates, was more effective and caused fewer adverse biochemical and tissue effects than linalool during short-term exposure.

Humans have used species from the mint family for medicinal purposes for over 2,000 years (Pittler and Ernst, 1998). Historical records show that ancient Egyptians, Romans, and Greeks utilized peppermint leaves to treat indigestion and relieve stomach pain. By the 18th century, doctors were prescribing peppermint extracts to help alleviate menstrual disorders in women (Keifer et al., 2007). Peppermint (*Mentha piperita*) is a hybrid species derived from spearmint (*M. spicata*) and watermint (*M. aquatica*). It has long been widely used as a medicinal plant in both Middle Eastern and Western cultures (Can and Sumer, 2019). Today, peppermint and its essential oil or extracts are employed across various industries, including food, perfumery, cosmetics, and pharmaceuticals, as herbal additives or aromatic agents (Foster, 1996). The primary active component of peppermint oil is menthol (McKay and Blumberg, 2006), a monoterpenoid compound known for its anesthetic effects. Owing to this property, peppermint extract (PE) has been proposed as a potential herbal anesthetic for application in aquaculture (Pittler and Ernst, 1998; Can and Sumer, 2019). For instance, Mazandarani and Hoseini (2016) found that menthol concentrations ranging from 118 to 512 mg/L induced general anesthesia in common carp (*Cyprinus carpio*) within 1 to 3 minutes of exposure. Their study also indicated that menthol concentrations below 600 mg/L did not cause mortality in fish. In addition to its anesthetic properties, PE has shown antiparasitic activity, particularly against monogenean worms (De Oliveira Hashimoto et al., 2016). While its effects have been well-documented in mammals, studies on the impact of PE in fish remain limited (Pittler and Ernst, 1998).

Recent studies have increasingly explored the physiological responses of fish to various anesthetics. For example, research has examined the effects of 2-phenoxyethanol on stress parameters in Atlantic salmon (*Salmo salar*) and the influence of dietary PE on growth and feed efficiency in aquaculture species (Mazandarani and Hoseini, 2018; Tarkhani et al., 2017). These investigations reflect a growing interest in enhancing fish health and welfare through both natural and synthetic agents. Among these, PE has received particular attention. Mazandarani and Hoseini (2018) evaluated its anesthetic efficacy and stress-related impacts in juvenile Persian sturgeon (*A. persicus*). Their study assessed behavioral responses, serum cortisol levels, and histopathological effects on gill and kidney tissues. Results showed that peppermint extract successfully induced anesthesia and increased stress hormone levels, without causing significant histopathological changes in the examined tissues. While their findings contribute valuable knowledge, they contrast with the current study, which focuses on a broader range of physiological and histological responses to varying concentrations of PE. Special emphasis is placed on its potential to mitigate stress and its tissue-level impacts. Toxicity testing is a common method used to evaluate the effects of various substances, including pesticides, pollutants, herbal extracts, and organic toxins, on aquatic organisms under controlled laboratory conditions (McKay et al., 2006; Yalsuyi et al., 2021b). Although this approach has limitations, it yields important information on compound toxicity, behavioral changes post-exposure, tissue damage, physiological responses, and overall organism health (Can and Sumer, 2019). Additionally, it offers insight into the potential ecological impacts of these substances in both natural and simulated environments (Mazandarani and Hoseini, 2018). This study aims to assess the behavioral, physiological, and histological responses of Persian sturgeon (*A. persicus*), a valuable species in aquaculture, following both short-term and long-term exposures to PE as a natural anesthetic. Given the anatomical complexity of the female sturgeon reproductive system, which often necessitates abdominal dissection and surgical egg extraction, the use of safe and effective anesthetics with minimal side effects is especially important (De Padua et al., 2010).

MATERIALS AND METHODS

All test procedures and sampling methods were developed and adapted based on the protocols outlined in the studies (Pittler & Ernst, 1998., Stoskopf and Posner, 2008., Hoseini et al., 2013., Mazandarani and Hoseini., 2018). Furthermore, all experimental conditions and testing protocols were approved by the Ethics Committee of Gorgan University of Agricultural Sciences and Natural Resources (GAU), in accordance with institutional animal welfare guidelines and experimental regulations (Approval No. 9521022-5e). These regulations were adapted from the technical report of the United States Environmental Protection Agency (EPA) Committee on Methods for Toxicity Tests with Aquatic Organisms, specifically concerning procedures for acute toxicity testing in fish, macroinvertebrates, and amphibians (Yalsuyi et al., 2021a).

Fish

For this experiment, 200 juvenile Persian sturgeons (*A. persicus*), with an average weight of 76.55 ± 2.17 g, were sourced from a local aquaculture facility (Shahid Marjani Fish Reproduction and Breeding Center, Golestan Province, Iran) and transported to the laboratory at Veniro Hall, Department of Fisheries, Faculty of Fisheries and Environmental Sciences, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.

To acclimate the fish to laboratory conditions, they were distributed among four fiberglass tanks, each with a dewatering capacity of 2,000 liters, and maintained for a two-week adaptation period. During this period, the fish were fed twice daily at a rate of approximately 3% of their body weight using commercial feed (BioMar Group, Aarhus, Denmark). Each tank was equipped with independent aeration, and 40% of the water was replaced daily to maintain optimal water quality. Water parameters, including pH, temperature, dissolved oxygen (DO), ammonia (NH_3) concentration, and total hardness (as $CaCO_3$), were monitored twice daily and adjusted as needed through aeration or water exchange. Measurements were taken using the following equipment: a digital soil and substrate pH meter (S500 Pro, Aqua Masters, Burbank, California, USA), a dissolved oxygen meter for aquaculture (HI9147, HANNA Instruments, Bertoki, Slovenia), and a multiparameter photometer (Model 7100, Palintest Co., Gateshead, UK). The mean values of the water's physicochemical properties during the acclimation period were as follows: temperature 23 ± 2 °C, dissolved oxygen 8 ± 1 mg/L, ammonia concentration < 0.01 mg/L, pH 7.6 ± 0.4, and total hardness 189 ± 6 mg/L $CaCO_3$. The photoperiod was maintained at 15 hours of light and 9 hours of darkness.

Anesthesia test

PE was procured from Adonis Gol Darou Co. (Tehran, Iran). According to the product factsheet, each milliliter of the extract contained between 5 and 5.7 mg of menthol. To facilitate better dissolution in water, the extract was first diluted in 99% ethanol at a ratio of 1:4. The solubility of the extract in absolute ethanol (≥ 99.9%, Merck, Darmstadt, Germany) exceeded 95%, with the solvent having a density of 0.79 g/cm³. Following the acclimation period, 90 juvenile Persian sturgeons were randomly selected and distributed into test tanks, with five fish assigned to each tank. The experiment comprised six treatment groups with three replicates each, using PE at concentrations of 0, 150, 250, 500, 750, and 1000 mg/L. To assess the potential effects of the ethanol solvent on experimental parameters, an additional 75 fish were exposed to ethanol concentrations of 450, 750, 1500, 2250, and 3000 mg/L. Water physicochemical parameters in the test tanks were consistent with those maintained during the adaptation phase. These included a temperature of 23 ± 2 °C, dissolved oxygen concentration of 8 ± 1 mg/L, ammonia concentration below 0.01 mg/L, pH of 7.6 ± 0.4, and total hardness of 189 ± 6 mg/L $CaCO_3$. Each fiberglass test tank had a dewatering capacity of 200 L and was equipped with water inlet and outlet systems (Mazandarani and Hoseini, 2018). Aeration was provided to maintain adequate oxygen levels. The experimental procedure was divided into two phases. In the first phase, fish were exposed to the treatment solutions for 20 minutes to induce anesthesia. Anesthetic depth was assessed using a five-stage scale (Table 1), with behavioral changes and induction times recorded for each stage. In the second phase, fish were transferred immediately, 20-minutes post-exposure, into new tanks containing fresh water with 0 mg/L of extract or ethanol. Recovery times were then recorded. The recovery tanks had the same dewatering volume (200 L) and physicochemical water parameters as the initial test tanks.

Blood sampling

To assess the physiological responses of the fish, blood samples were collected from five individuals per treatment group at stage 5 of anesthesia. Sampling was conducted via the caudal vein at three time points: immediately after anesthesia (0 hours), and at 24 and 72-hours post-anesthesia. Both heparinized and non-heparinized syringes were used to analyze hematocrit (Hct), hemoglobin concentration (Hb), total erythrocyte count (TEC), and total leukocyte count (TLC). Hct was determined using the microhematocrit centrifugation method. Samples were centrifuged at 10,000 rpm for 7 minutes using a microcentrifuge (D-78532 Tuttlingen, Hettich, Germany), and values were expressed as a percentage of total blood volume. Hb concentration was measured using the Cyanomethemoglobin method with a UV/Vis spectrophotometer (Jenway 6505, United Kingdom) at an absorbance of 540 nm. TEC and TLC were assessed using a Neubauer hemocytometer and Daice diluting solution. Differential white blood cell (WBC) counts were determined from Giemsa-stained blood smears and examined under a light microscope (Olympus, Tokyo, Japan) (Yalsuyi et al., 2021a; Mazandarani and Hoseini, 2018).

Table 1. Juvenile Persian sturgeon (*Acipenser persicus*) behaviors at different anesthesia stages with peppermint extract and ethanol

Stage	Exhibited behavior
0	Normal
I	Rapid movements, excitation, and saltation
II	Lethargy and normal gills movements, no swimming
III	Spiral and imbalance swimming, gill movements
IV	Loss of equilibrium, pectoral fin rapid movements, and irregular gill movements
V	significant decrease in gill movement frequency
Recovery	Fish regained its equilibrium.

Note: all parameters were selected according to Hoseini et al. (2013) study and modified.

Blood sampling

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Japan) (Yalsuyi et al., 2021b; Mazandarani and Hoseini, 2018).

Tissue sampling

Tissue samples were dissected from the second arch of the right gill and the middle portion of the liver immediately after euthanizing three fish from each treatment group. All samples were fixed in 4% neutral phosphate-buffered formalin (pH 7.2), with the fixative renewed after 12 hours to ensure optimal preservation. After fixation, the samples underwent a dehydration process to facilitate paraffin infiltration. This involved sequential immersion in a graded ethanol series (80%, 90%, 97%, and 100%) to effectively remove interstitial water and residual fixative. Following dehydration, xylene was used as a clearing agent to replace the ethanol, minimizing tissue shrinkage and distortion. To preserve the natural morphology of the tissues, they were embedded in paraffin using a mixture of xylene and soft paraffin in a 1:4 ratio. The paraffin-embedded samples were then transferred to aluminum molds and allowed to cool and solidify prior to sectioning. Serial sections, each 5 µm thick, were prepared using an ultra-microtome (Olympus CUT 4055E, USA). The sections were mounted onto glass slides that had been pre-cleaned by immersion in 70% ethanol. After cleaning, the slides were coated with a mounting medium and left to dry completely. Tissue sections were floated on water, carefully lifted at a 45-degree angle, and labeled with a diamond pencil. Staining was performed using the hematoxylin and eosin (H&E) method, the standard protocol for histological analysis. After staining, the slides were covered with glass coverslips that had been dried with methanol to prevent moisture interference. A drop of Canada balsam was applied to the stained sections before placing the coverslips, ensuring complete adhesion and eliminating air bubbles. Finally, the prepared slides were examined under a 1000× optical microscope based on established histopathological criteria for tissue lesion identification. Photomicrographs were taken for detailed anatomical analysis (Hoseini et al., 2013; Hedayati et al., 2013).

Data analysis

To estimate the differential leukocyte count, blood smears were prepared, air-dried, fixed in methanol, and stained with May–Giemsa solution. Leukocytes in the smears were classified into lymphocytes, monocytes, neutrophils, and eosinophils (Mazandarani and Hoseini, 2016). The Shapiro–Wilk test was used to assess data normality and homogeneity of variance. Mean induction and recovery times across anesthetic concentrations were compared using one-way ANOVA followed by Duncan's test to identify significant differences ($P < 0.05$). Data are reported as mean ± standard deviation (SD). Statistical analyses were performed using SPSS software (version 21.0).

RESULTS

No fish mortality was observed during the adaptation period or the experiment. The test results were as follows:

Anesthesia effect

All tested concentrations of PE successfully induced anesthesia in juvenile Persian sturgeon (*A. persicus*). A significant inverse correlation was observed between oil concentration and induction time ($P < 0.01$): fish exposed to 150 mg/L had the longest induction time, while those treated with 1000 mg/L reached full anesthesia the fastest. At all concentrations, induction occurred within six minutes. Recovery times increased proportionally with the concentration of the extract. The shortest recovery was recorded at 150 mg/L, and the longest at 1000 mg/L. Fish exposed to concentrations between 300 and 500 mg/L demonstrated the most balanced anesthetic response, with both induction and recovery times falling within the optimal range of 3–5 minutes (Table 2). A significant positive correlation was also found between oil concentration and recovery time ($P < 0.01$). In general, higher concentrations of the herbal oil extract reduced induction time but extended recovery time. The smallest gap between induction and recovery durations occurred at concentrations of 300–500 mg/L (Fig. 1). Ethanol, used as a control, did not induce anesthesia or alter behavior at any tested concentration. Fish in the ethanol group exhibited normal behavior and physiology throughout the exposure period. Clinical signs typically associated with anesthetic induction, such as excitation, lethargy, abnormal gill movements, spiral or unbalanced swimming, a marked decrease in gill movement frequency, and loss of equilibrium, were not observed in these fish. Moreover, no mortality was recorded during the experiment.

Hematological parameters

Analysis of blood samples revealed no significant differences in hematological parameters between the control and ethanol-treated groups 24 hours after exposure (Table 3). However, fish exposed to PE concentrations above 500 mg/L showed significantly elevated (Hct), Hb, and RBC counts compared to the control group ($P < 0.05$). In contrast, no significant changes were observed in WBC counts across any treatment groups. For fish exposed to PE concentrations below 500 mg/L, hematological parameters did not differ significantly from those of the control group ($P < 0.05$). Similarly, Table 4 indicates no significant differences in hematological indices between the control and peppermint oil-treated groups at these lower concentrations. Importantly, all observed differences had resolved by 72-hours post-exposure. At that time point, hematological parameters in all treatment groups were comparable to those in the control group, with no statistically significant differences.

Tissue pathology

No significant histopathological alterations, such as hyperplasia, hepatocyte vacuolization, or tubular necrosis, were observed in the gills, liver, or kidneys of fish in the control group. In contrast, all concentrations of PE induced varying degrees of tissue damage. In the gills, the most common pathological changes included hyperplasia, epithelial lifting, and congestion in the secondary lamellae across all exposure levels (Fig. 2). Severe hyperplasia at the base of the secondary lamellae was particularly evident at the highest concentrations (250 and 500 mg/L). Liver tissues of fish exposed to lower concentrations of PE (150,

250, and 500 mg/L) appeared normal, with no observable pathological changes. However, at higher concentrations (750 and 1000 mg/L), notable hepatic damage was detected, including hepatocyte degeneration and vacuolization (Fig. 3). The kidneys showed concentration-dependent pathological effects. At higher extract concentrations (500, 750, and 1000 mg/L), the most prominent alterations included renal tubular degeneration and dilation, often accompanied by eosinophilic casts (Fig. 4).

Table 2. Induction of anesthesia and recovery times of juvenile Persian sturgeon (*Acipenser persicus*) exposed to the peppermint (*Mentha piperita*) extract

Anesthesia level	Concentration of peppermint extract (mg/L)				
	150	250	500	750	1000
	Induction anesthesia time (sec)				
Stage I	102.91± 45.50^{a}	56 ± 22.58 ^{b}	53.33 ± 25.18^{b}	48.15 ± 24.50^{b}	46.66 ± 21.36 ^{b}
Stage II	132.91 ± 40.9 ^{a}	86.3 ±29.65 ^{b}	84.66 ± 31.8 ^{b}	76.92 ± 28.39 ^{b}	71.66 ± 43.2 ^{b}
Stage III	167.91 ± 41.6 ^{a}	155 ± 48.53 ^{a}	117.66 ± 44.7 ^{b}	108.46 ± 41.40^{b}	102.5± 40.45 ^{b}
Stage IV	247 ± 59.49 ^{a}	227 ± 50.11^{a}	168.66 ± 44.8 ^{b}	129.23 ± 42.07^{bc}	117.5±43.67 ^{c}
Stage V	370.5 ± 96.3 ^{c}	319 ± 50.24 ^{a}	197.57 ± 44 ^{b}	155.76 ± 45.40^{bc}	145.8 ± 50.6 ^{c}
	Recovery time (sec)				
Stage I	28.33±21.35^{c}	34.66±16.74^{c}	67.27±11.05^{b}	88.75±13.90^{a}	93.63±11.20^{a}
Stage II	56.66±28.55^{c}	72.33±27.33^{c}	157.5±47.36^{b}	160±37.26^{b}	352.5±35.8^{a}
Stage III	82.99±25.89^{d}	124.66±45.13^{c}	223.63±33.47^{b}	227.72±29.15^{b}	432.5±21.8^{a}
Stage IV	102.5±21.44^{d}	164.66±15.13^{c}	269.36±19.2^{b}	271.81±39.78^{b}	477.5±34.3^{a}
Stage V	171.5±19.59^{d}	243.07±12.14^{c}	290±30.36^{b}	388.18±22.69^{b}	537.5±43.8^{a}

Note: Different superscript letters showed significant differences between the contents of the same rows ($P < 0.05$).

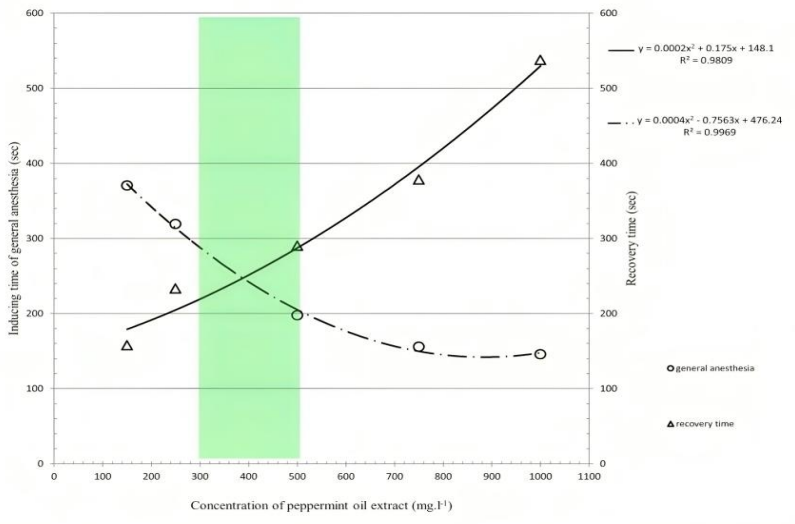

Fig. 1. A significant correlation ($P < 0.01$) between the concentration of the peppermint extract, *Mentha piperita* induction time of general anesthesia and recovery times of juvenile Persian sturgeon (*Acipenser persicus*). The lowest differences between the induction time of general anesthesia and recovery time were in the range of 300 to 500 mg/L (the green zone).

Table 3. Effect of different concentrations of ethanol on hematological parameters of juvenile Persian sturgeon (*Acipenser persicus*) 24 hours after exposure

Parameter	Control	Concentration of ethanol (mg/L)				
		450	750	1500	2250	3000
Hematocrit (%)	26.48 ± 0.60^{c}	27.2 ± 1.47^{a}	26.45 ± 2.60^{a}	27.33 ± 2.42^{a}	26.52 ± 2.06^{a}	27.21 ± 2.22^{a}
Hemoglobin (g dL^{-1})	7.92 ± 0.89^{a}	7.88 ± 0.73^{a}	7.90 ± 0.75^{a}	7.91 ± 0.29^{a}	7.86 ± 0.95^{a}	7.89 ± 0.15^{a}
Red Blood Cell (× 10^{6} cell/m^{3})	6.67 ± 0.28^{a}	6.64 ± 0.1^{a}	6.67 ± 0.29^{a}	6.68 ± 0.39^{a}	6.67 ± 0.34^{a}	6.65 ± 0.69^{a}
White Blood Cell (× 10^{4} cell/m^{3})	1.21 ± 0.22^{a}	1.22 ± 0.26^{a}	1.22 ± 0.25^{a}	1.24 ± 0.52^{a}	1.21 ± 0.24^{a}	1.22 ± 0.20^{a}
Lymphocytes (%)	51.55 ± 3.35^{a}	52.52 ± 3.05^{a}	51.48 ± 5.11^{a}	52.02 ± 3.77^{a}	51.63 ± 8.20^{a}	51.93 ± 8.20^{a}
Monocytes (%)	0.91 ± 0.49^{a}	0.93 ± 0.75^{a}	0.92 ± 0.55^{a}	0.91 ± 0.49^{a}	0.90 ± 0.79^{a}	0.90 ± 0.59^{a}
Neutrophils (%)	46.66 ± 3.54^{a}	45.65 ± 3.37^{a}	46.33 ± 2.69^{a}	46.16 ± 3.62^{a}	46.58 ± 1.93^{a}	46.28 ± 1.91^{a}
Eosinophils (%)	0.88 ± 0.15^{a}	0.90 ± 0.15^{a}	0.91 ± 0.22^{a}	0.91 ± 0.19^{a}	0.89 ± 0.18^{a}	0.89 ± 0.18^{a}

Note: Different superscript letters showed significant differences between the contents of the same rows ($P < 0.05$).

Table 4. Effect of different concentrations of peppermint extract on hematological parameters of juvenile Persian sturgeon (*Acipenser persicus*) 24 hours after exposure

Parameter	Control	Concentration of peppermint extract (mg/L)				
		150	250	500	750	1000
Hematocrit (%)	26.48 ± 0.60^{d}	27.46 ± 0.47^{cd}	27.35 ± 0.21^{d}	28.19 ± 0.32^{b}	31.82 ± 0.13^{a}	31.62 ± 0.70^{a}
Hemoglobin (g dL^{-1})	7.92 ± 0.89^{b}	7.89 ± 0.13^{b}	7.91 ± 0.25^{b}	7.94 ± 0.39^{b}	8.98 ± 0.15^{a}	9.01 ± 0.35^{a}
Red Blood Cell ($\times 10^{6}$ cell/m^{3})	6.67 ± 0.58^{b}	6.67 ± 0.36^{b}	6.68 ± 0.17^{b}	6.70 ± 0.33^{b}	7.83 ± 0.37^{a}	7.85 ± 0.29^{a}
White Blood Cell ($\times 10^{4}$ cell/m^{3})	1.21 ± 0.22^{a}	1.22 ± 0.31^{a}	1.21 ± 0.28^{a}	1.23 ± 0.42^{a}	1.23 ± 0.24^{a}	1.22 ± 0.41^{a}
Lymphocytes (%)	51.55 ± 3.35^{a}	51.61 ± 2.05^{a}	51.65 ± 2.21^{a}	52.92 ± 1.77^{a}	51.89 ± 1.20^{a}	52.08 ± 2.48^{a}
Monocytes (%)	0.91 ± 0.49^{a}	0.90 ± 0.85^{a}	0.89 ± 0.75^{a}	0.91 ± 0.19^{a}	0.92 ± 0.09^{a}	0.90 ± 0.39^{a}
Neutrophils (%)	46.66 ± 3.54^{a}	46.61 ± 1.37^{a}	46.33 ± 0.64^{a}	45.32 ± 2.62^{a}	45.28 ± 1.03^{b}	46.14 ± 0.92^{a}
Eosinophil (%)	0.88 ± 0.15^{a}	0.88 ± 0.19^{a}	0.87 ± 0.13^{a}	0.88 ± 0.06^{a}	0.84 ± 0.53^{a}	0.88 ± 0.11^{a}

Note: Different superscript letters showed significant differences between the contents of the same rows ($P < 0.05$).

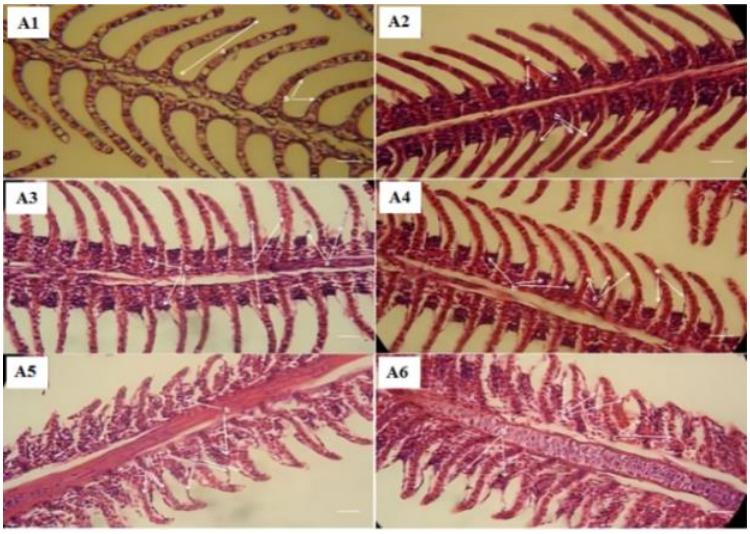

Fig. 2. Gill tissue of juvenile Persian sturgeon (*Acipenser persicus*) following exposure to various concentrations of peppermint (*Mentha piperita*) extract: (A1) Control group showing normal gill structure, including (a) secondary lamellae and (b) pillar cells; (A2) 150 mg/L: (a) mild hyperplasia at the base of secondary lamellae and (b) congestion in the secondary lamellae; (A3) 250 mg/L: (a) epithelial lifting, (b) mild hyperplasia at the base of secondary lamellae, and (c) congestion in the secondary lamella; (A4) 500 mg/L: (a) epithelial lifting, (b) mild hyperplasia at the base of secondary lamellae, and (c) congestion in the secondary lamella; (A5) 750 mg/L: (a) epithelial lifting, (b) severe hyperplasia at the base of secondary lamellae, and (c) congestion in the secondary lamella; (A6) 1000 mg/L: (a) epithelial lifting, (b) severe hyperplasia at the base of secondary lamellae, and (c) congestion in the secondary lamella. Scale bar = 100 µm.

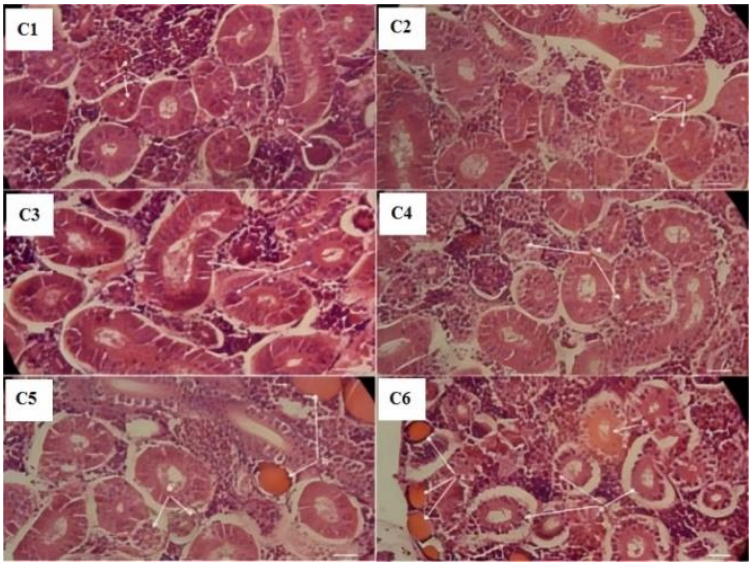

Fig. 3. The liver tissue of Persian sturgeon juvenile (*Acipenser persicus*) after exposure to different concentrations of peppermint (*Mentha piperita*) extract: (B_1) the control group, (a) normal structure hepatocytes and (b) central vein; (B_2) (a) normal structure hepatocytes and (b) central vein (150 mg/L); (B_3) (a) normal structure hepatocytes and (b) central vein (250 mg/L); (B_4) (a) normal structure hepatocytes and (b) central vein (500 mg/L); (B_5) (a) mild hepatocytes vacuolization (750 mg/L); B_6): (a) mild degeneration and vacuolization of hepatocytes (100 mg/L). (bar = 100 µm).

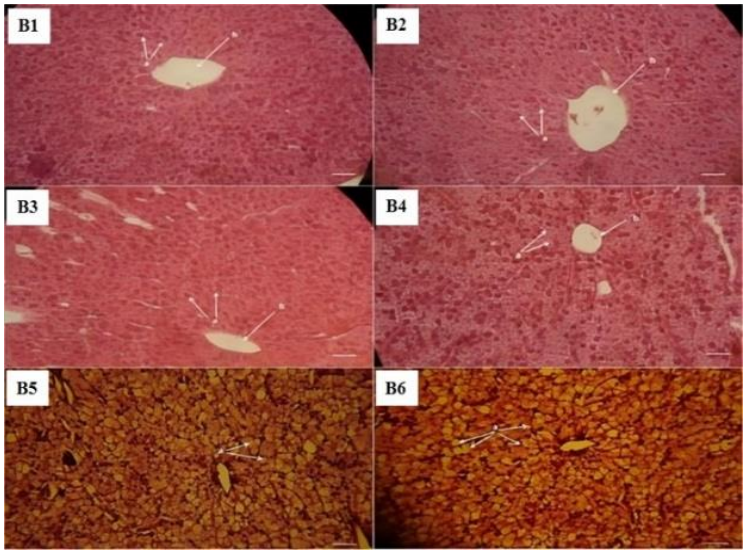

Fig. 4. The kidney tissue of Persian sturgeon juvenile (*Acipenser persicus*) after exposure to different concentrations of peppermint (*Mentha piperita*) extract: (C_1) the control group, (a) the normal structur of kidney tubules and (b) central vein; (C_2) (a) the normal structure of kidney tubules (150 mg/L); (C_3) (a) the normal structure of kidney tubules (250 mg/L (C_4) (a) local degeneration in some kidney tubules (500 v); (C_5) (a) mild degeneration in some kidney tubules, (b) dilated renal tubules with the eosinophilia cast (750 mg/L); (C_6) (a) mild degeneration in some kidney tubules, (b) tubules necrosis., and (c) dilated renal tubules with the eosinophilia cast (1000 mg/L). (bar = 100 µm).

DISCUSSION

The findings of this study demonstrate that PE can be an effective anesthetic for juvenile Persian sturgeon (*A. persicus*), especially at concentrations between 300 and 500 mg/L, where favorable induction and recovery times were observed. However, higher concentrations (750–1000 mg/L) led to adverse physiological and histopathological effects, highlighting the importance of precise dosage control. A comparison with the study by Mazandarani and Hoseini (2018) reveals notable differences. While their research reported no histopathological changes in gill or kidney tissues at comparable concentrations, the present study observed significant tissue damage at higher doses. These discrepancies may stem from differences in experimental conditions, including fish size, duration of exposure, and the composition of the PE used. The use of a more concentrated PE in the current study could also account for the observed effects, underscoring the need for standardized methodologies in future investigations. The hematological changes observed, specifically increases in Hct, Hb, and RBC counts, are consistent with previous findings on the stress-inducing effects of menthol-based anesthetics. Although these parameters returned to baseline within 72 hours, they suggest that short-term physiological stress can occur during exposure. This supports earlier research indicating that menthol compounds may activate stress-related pathways in fish, even at anesthetic levels.

Histopathological analysis further emphasizes the dual role of PE as both an effective anesthetic and a potential physiological stressor. While low to moderate concentrations caused only minor tissue alterations, higher concentrations resulted in severe damage, particularly in the gill and kidney tissues. These findings underscore the importance of balancing efficacy and safety when considering PE for use in aquaculture. Given the growing interest in herbal anesthetics for fish—driven by their perceived health benefits (Pittler and Ernst, 1998)—it is essential to thoroughly assess their impacts on fish health. The present study evaluated the effects of various concentrations of peppermint extract on histological and hematological parameters, behavior, anesthesia induction, and recovery time in Persian sturgeon. Determining the optimal anesthetic concentration for different fish species is essential, as inappropriate dosages can lead to negative outcomes, including elevated stress levels (Can and Sumer, 2019).

Anesthetics are commonly used in aquaculture to minimize stress responses during fish handling. However, the administration of anesthesia itself can trigger a stress response (Foster, 1996). Therefore, an ideal anesthetic should induce anesthesia quickly and with minimal physiological disturbance, preferably within 3 to 5 minutes, to reduce stress and prevent excessive agitation. Additionally, recovery should be swift, with fish regaining normal function within 5 minutes after being transferred to aerated water (Moghim et al., 2006; Chambel et al., 2013). The anesthetic properties of PE have been minimally explored in aquaculture contexts. In the present study, PE was found to induce excitation in *A. persicus*, likely due to the detection of its chemical constituents by the olfactory and gustatory systems (Can and Sumer, 2019), as well as the stimulation of cold-sensitive nociceptors (De Oliveira Hashimoto et al., 2016; Hoseini et al., 2010). Fish exposed to various concentrations of the extract, particularly during stage 1 anesthesia, exhibited excitatory behavior, which was more pronounced at higher concentrations. This reaction may be due to the initial suppression of inhibitory neurons prior to the onset of full anesthesia (Hunn and Allen, 1974). Kasai et al. (2014) reported that higher concentrations of menthol (469 mg/L) provoked rapid movement and

pain-related responses in fish, whereas lower concentrations (78.1–312.5 mg/L) did not. While limited data are available on the broader physiological, hematological, and histopathological effects of *M. piperita* on fish, several studies have investigated menthol, the main active compound in essential oils derived from *Mentha* species.

Façanha and Gomes (2005) evaluated the anesthetic efficacy of menthol in tambaqui (*Colossoma macropomum*) and found that a concentration of 150 mg/L induced deep anesthesia (stage 4), characterized by reduced and irregular gill movements. They reported induction and recovery times of 130 and 660 seconds, respectively, concluding that menthol could be a viable alternative to benzocaine and eugenol. Similarly, De Padua et al. (2010) studied menthol concentrations ranging from 60 to 150 mg/L in dourado (*Salminus brasiliensis*) and found all tested levels to be effective in producing anesthesia. Kasai et al. (2014) demonstrated that menthol at concentrations of 46.8, 62.4, 78, and 156 mg/L induced stage 4 anesthesia in medaka (*Oryzias latipes*) within 300, 250, 200, and 150 seconds, respectively, with corresponding recovery times of 300, 350, 500, and 650 seconds. Pedrazzani and Neto (2014) investigated the effects of *Mentha arvensis* in clown anemonefish (*Amphiprion ocellaris*), finding that 72.8 mg/L induced deep anesthesia within 312 seconds, followed by recovery in 329.5 seconds. These findings highlight interspecies variation in sensitivity to mint-derived anesthetics. Optimal menthol concentrations also vary by species. For example, the recommended concentration for pacu (*Piaractus mesopotamicus*) is 100 mg/L (Gonçalves et al., 2008), while for *C. macropomum*, Façanha and Gomes (2005) suggested 150 mg/L. Teixeira et al. (2011) reported that juvenile and fingerling Nile tilapia (*Oreochromis niloticus*) required 120 and 60 mg/L, respectively, to achieve deep (surgical) anesthesia. Another study on *O. niloticus* fingerlings found that PE at concentrations of 40–160 mg/L induced anesthesia lasting 1200–1300 seconds (Yalsuyi et al., 2021a). Menthol concentrations between 50 and 500 mg/L anesthetized common carp (*Cyprinus carpio*) fingerlings within 350–353 seconds, with recovery occurring over 180–1000 seconds (De Padua et al., 2010). In lambari (*Astyanax altiparanae*) fry, menthol at 50–300 mg/L induced deep anesthesia within 32–62 seconds, with recovery times ranging from 110 to 250 seconds (Pereira-da-Silva et al., 2014). Additionally, menthol at 100–800 mg/L was found to anesthetize giant river prawn (*Macrobrachium rosenbergii*) within approximately 210 seconds, with recovery occurring between 78 and 150 seconds (Saydmohammed and Pal, 2009).

In the present study, increasing concentrations of PE (50–333 mg/L) were associated with shorter induction times but longer recovery durations. This variation in anesthesia and recovery times likely reflects individual behavioral differences among the experimental fish. Specifically, anesthesia was achieved in Persian sturgeon (*A. persicus*) at concentrations of 150–1000 mg/L within 102–477 seconds, with recovery occurring between 171 and 537 seconds. Histological analysis is a crucial tool for identifying tissue damage caused by anesthetic agents (Roberts, 2012). The gills, which are central to anesthetic uptake during immersion (Hunn and Allen, 1974), also regulate critical biological functions (Evans et al., 2005) and are key indicators of fish welfare (Pettersen et al., 2014). Consequently, monitoring gill health is essential during anesthesia exposure. Prior studies have reported capillary ectasia in gill filaments following exposure to various anesthetics, including 2-phenoxyethanol in sheatfish (*Silurus glanis* L.) (Velisek et al., 2007), clove oil in common carp (*Cyprinus carpio*) (Velisek et al., 2005), and benzocaine in melon barb (*Haludaria fasciata*) (Padiyoor et al., 2017). Benzocaine and eugenol also induced similar gill effects in *Colossoma macropomum*, without pathological changes in other tissues (Boijink et al., 2017). In contrast, Posner et al. (2013) found no histomorphological alterations in the gills of *Carassius auratus* following repeated exposure to 160 mg/L MS-222. However, Gomulka et al. (2008) reported swelling of the primary and secondary lamellae due to the epithelial cell hypertrophy in Siberian sturgeon (*Acipenser baerii*) after 24-hour exposure to 125 mg/L MS-222. Similarly, Chance et al. (2018) observed epithelial lifting from lamellar capillaries in Atlantic salmon (*Salmo salar*) anesthetized with MS-222 and AQUI-S. In common carp, clove oil anesthesia also caused capillary ectasia in gill filaments immediately post-exposure, though no histopathological changes were noted in other tissues, such as the liver, spleen, or kidneys (Velisek et al., 2005). In the current study, exposure to *M. piperita* resulted in histopathological damage across multiple organs. The gills showed signs of hyperplasia, congestion, and epithelial lifting in the secondary lamellae. In the liver, hepatocyte degeneration and vacuolization were observed at higher concentrations (750–1000 mg/L). Renal damage included degeneration of some renal tubules, dilation with eosinophilic casts, and tubular necrosis. These findings indicate that while *M. piperita* is effective as an anesthetic, higher concentrations pose significant risks to fish health. This reinforces the importance of accurate dosage regulation in aquaculture practices to ensure both efficacy and safety.

Fish exposed to various stressors or treatments often show notable hematological changes. Hct reflects the proportion of RBCs relative to WBCs and plasma. RBCs are responsible for oxygen and carbon dioxide transport and are produced in hematopoietic tissues located primarily in the spleen and head kidney (Chambel et al., 2013; Posner et al., 2013). In the present study, the combination of increased hematocrit levels alongside decreased RBC counts suggests possible stress or respiratory dysfunction, which may underlie the reduced RBC numbers observed. Supporting this, De Oliveira Hashimoto et al. (2016) reported that *M. piperita* exposure increased hemoglobin content as well as erythrocyte and monocyte levels. Consistently, this study found significant elevations in Hct and Hb following anesthesia with *M. piperita*. It is well established that decreases in both the quantity and quality of erythrocytes, together with reduced hemoglobin levels, impair oxygen delivery to tissues.

A non-significant difference ($P > 0.05$) was observed in the erythrocyte count of juvenile Persian sturgeon exposed to various concentrations of peppermint extract compared to the control group. This contrasts with findings by De Oliveira Hashimoto et al. (2016), who reported an increase in erythrocyte count in Nile tilapia following exposure to *M. piperita*. To evaluate the impact of *M. piperita* on the fish immune system, WBC counts were also measured. The present study found no significant changes in total WBC or differential leukocyte counts. Wedemeyer et al. (1990) noted that stress during anesthesia can influence WBC and differential leukocyte profiles. For instance, Barcellos et al. (2004) documented alterations in WBC and leukocyte differentials in *Rhamdia quelen* under acute and chronic stress. However, other anesthesia studies have reported no significant changes in these hematological parameters (Mohammadizarejabad et al., 2009; Weber et al., 2009). In contrast, Imanpoor et al. (2010) observed a decline in WBC counts during clove essence anesthesia in Persian sturgeon. In summary, this study provides valuable insights into the anesthetic potential and limitations of PE in aquaculture. Future research should aim to refine optimal concentration ranges, investigate long-term effects, and standardize experimental protocols to improve comparability across studies. Additionally, further exploration of the biochemical pathways mediating stress responses induced by PE could deepen our understanding of its physiological impacts on fish health.

CONCLUSION

Application of herbal anesthetics in aquaculture is increasingly becoming prevalent due to their natural origin and potential benefits. This study evaluated the pathological effects of PE on *A. persicus*. While peppermint extract exhibited effective anesthetic properties, it also induced physiological changes that raise important animal welfare concerns, an essential consideration in laboratory and aquaculture practices. Consequently, peppermint may not be an ideal anesthetic for Persian sturgeon. Nevertheless, peppermint is widely used in food products and is generally considered safe for human consumption, posing no significant health risks.

CRediT AUTHORSHIP CONTRIBUTION STATEMENT

Conceptualization: Mohammad Mazandarani and Mohammad Sudagar; Methodology: Mohammad Mazandarani and Mohammad Sudagar; Supervision: Mohammad Mazandarani; Validation: Mohammad Mazandarani; Formal analysis: Sara Vali and Mohammad Mazandarani; Investigation: Mohammad Reza Imanpour; Data curation: Sara Vali; Writing—original draft preparation: Sara Vali; Writing—review and editing: Mohammad Mazandarani and Mohammad Sudagar; Project administration: Mohammad Sudagar and Mohammad Reza Imanpour; Funding acquisition: Mohammad Mazandarani.

DECLARATION OF COMPETING INTEREST

The authors declare no conflicts of interest.

ETHICAL STATEMENT

All experimental conditions and procedures were approved by the Ethical Committee of Gorgan University of Agricultural Sciences and Natural Resources (GAU) and conducted in accordance with established animal welfare guidelines and experimental protocols. These protocols were adapted from the technical report by the Committee on Methods for Toxicity Tests with Aquatic Organisms of the United States Environmental Protection Agency (EPA), which provides standardized methods for acute toxicity testing with fish, macroinvertebrates, and amphibians.

DATA AVAILABILITY

Not applicable.

ACKNOWLEDGMENTS

The authors sincerely thank the staff of Gorgan University of Agricultural Sciences and Natural Resources for their invaluable support and assistance throughout the course of this experiment.

REFERENCES

Barcellos, L. J. G., Kreutz, L. C., De-Souza, C., Rodrigues, L. B., Fioreze, I., Quevedo, R. M., Cericato, L., Soso, A. B., Fagundes, M., Conrad, J., Lacerda L. d. A., & Terra, S. (2004). Hematological changes in jundiá (*Rhamdia quelen* Quoy and *Gaimard Pimelodidae*) after acute and chronic stress caused by usual aquacultural management, with emphasis on immunosuppressive effects. *Aquaculture*, *237*, 229-236. https://doi.org/10.1016/j.aquaculture.2004.03.026

Bodura, T., Afonsoa, J. M., Monteroa, D., & Navarro, A. (2018). Assessment of effective dose of new herbal anesthetics in two marine aquaculture species: *Dicentrarchus labrax* and *Argyrosomus regius*. *Aquaculture*, *482*, 78-82. https://doi.org/10.1016/j.aquaculture.2017.09.029

Boijink, C. L., Maciel, P. O., Tavares-Dias, M., Iwashitab, M. K. P., Morais, M. S., Hidee, D. M. V., Souzaf, N. C., Couto, M. V. S., Meneses, J. O., Cunha, F. S., & Fujimoto, R. Y. (2017). Anesthesia by sprinkling method in the gills of Tambaqui (*Colossoma macropomum*) does not influence intensity and morphology of monogeneans. *Brazilian Journal of Biology*, *77*, 367-371. https://doi.org/10.1590/1519-6984.15915

Can, E., & Sumer, E. (2019). Anesthetic and sedative efficacy of peppermint (*Menthapiperita*) and lavender (*Lavandulaangustifolia*) essential oils in blue dolphin cichlid (*Cyrtocaramoorii*). *Turkish Journal of Veterinary & Animal Sciences*, *43*, 334-341. https://doi.org/10.3906/vet-1809-22

Chambel, J., Pinho, R., Sousa, R., Ferreira, T., Baptista, T., Severiano, V., Mendes, S., & Pedrosa, R. (2013). The efficacy of MS-222 as anaesthetic agent in four

freshwater aquarium fish species. *Aquaculture Research*, *46*(7), 1582-1589. https://doi.org/10.1111/are.12308

Chance, R. J., Cameron, G. A., Fordyce, M., Noguera, P., Wang, T., Collins, C., Secombes, C. J., & Collet, B. (2018). Effects of repeated anesthesia on gill and general health of Atlantic salmon, *Salmo salar*. *Journal of Fish Biology*, *93*, 1069-1081. https://doi.org/10.1111/jfb.13803

Coyle, S. D., Durborow, R. M., & Tidwell, J. H. (2004). *Anesthetics in aquaculture*. Texas, US: Southern Regional Aquaculture Center (SRAC) Publication.

De Oliveira Hashimoto, G. S., Neto, F. M., Ruiz, M. L., Acchile, M., Chagas, E. C., Chaves, F. C. M., & Martins, M. L. (2016). Essential oils of Lippia sidoides and Mentha piperita against monogenean parasites and their influence on the hematology of Nile tilapia. *Aquaculture*, *450*, 182-186. https://doi.org/10.1016/j.aquaculture.2015.07.029

De Padua, S. B., de Pietro, P. S., Iglessias-Filho, P. S., Ishikawa, M. M., & Hisano, H. (2010). Menthol as anesthesic for dourado (*Salminus brasiliensis*). *Boltim Do Instituto De Pesca*, *36*(2), 143-148.

Elumalaia, P., Kurian, A., Lakshmi, S., Faggio, C., Esteban, M. A., & Ringo, E. (2020). Herbal immunomodulators in aquaculture. *Reviews in Fisheries Science & Aquaculture*, *29*(1), 33-57. https://doi.org/10.1080/23308249.2020.1779651

Evans, D. H., Piermarini, P. M., & Choe, K. P. (2005). The multifunctional fish gill: Dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. *Physiological Reviews*, *85*, 97-177. https://doi.org/10.1152/physrev.00050.2003.

Façanha, M. F., & Gomes, L. D. C. (2005). Efficacy of menthol as an anesthetic for tambaqui (*Colossoma macropomum*, *Characiformes: Characidae*). *Acta Amazonica*, *35*, 71-75. https://doi.org/10.1590/S0044-59672005000100011

Faggio, C., Piccione, G., Marafioti, S., Arfuso, F., Fortino, G., & Fazio, F. (2014). Metabolic response to monthly variations of sparus aurata reared in mediterranean on-Shore tanks. *Turkish Journal of Fisheries and Aquatic Sciences*, *14*, 567-574. https://doi.org/10.4194/1303-2712-v14_2_28

Foster, S. (1996). *Peppermint: Mentha piperita*. USA: *American Botanical Council-Botanical Series*.

Gaikowski, M. P., Gingerich, W. H., & Gutreuter, S. (2001). Short-duration electrical immobilization of lake trout. *North American Journal of Fisheries Management*, *21*, 381-392. https://doi.org/10.1577/1548-8675(2001)021<0381:SDEIOL>2.0.CO;2

Gomulka, P., Własow, T., Velíšek, J., Svobodová, Z., & Chmielinska, E. (2008). Effects of eugenol and MS-222 anaesthesia on Siberian Sturgeon *Acipenser baerii* Brandt. *Acta Veterinaria Brno*, *77*, 447-453. https://doi.org/10.2754/avb200877030447

Gonçalves, A. F. N., Santos, E. C. C., Fernandes, J. B. K., & Takahashi, L. S. (2008). Mentol e eugenol como substitutos da benzocaína na indução anestésica de juvenis de pacu. *Acta Scientiarum. Animal sciences*, *30*, 339-344. https://doi.org/10.4025/actascianimsci.v30i3.1081

Hedayati, A. A., Jahanbakhshi, A. R., & Ghaderi-Ramazi, F. (2013). The effect of different silver nanoparticles on thyroid oils, growth, blood indices of body enzymes of common carp (*Cyprinus carpio*). *Aquatic Toxicology*, *68*, 74-82. https://doi.org/10.22124/cjes.2020.4138

Holloway, A. C., Keene, J. L., Noakes, D. G., & Moccia, R. D. (2004). Effects of clove oil and MS-222 on blood hormone profiles in rainbow trout *Oncorhynchus mykiss*, Walbaum. *Aquaculture Research*, *35*, 1025-1030. https://doi.org/10.1111/j.1365-2109.2004.01108.x

Hoseini, S. M., & Ghelichpour, M. (2012). Efficacy of clove solution on blood sampling and hematological study in Beluga, *Huso huso (L.)*. *Fish Physiology and Biochemistry*, *38*, 493-498. https://doi.org/10.1007/s10695-011-9529-5

Hoseini, S. M., Hosseini, S. A., & Nodeh, A. J. (2010). Serum biochemical characteristics of Beluga, *Huso huso (L.*), in response to blood sampling after clove powder solution exposure. *Fish Physiology and Biochemistry*, *37*, 567-572. https://doi.org/10.1007/s10695-010-9458-8

Hoseini, S. M., Rajabiesterabadi, H., & Tarkhani, R. (2013). Anesthetic efficacy of eugenol on the iridescent shark, *Pangasius hypothalamus* (Sauvage, 1878) in different size classes. *Aquaculture Research*, *46*, 405-412. https://doi.org/10.1111/are.12188

Hunn, J. B., & Allen, J. L. (1974). Movement of drugs across the gills of fishes. *Annual Review of Pharmacology and Toxicology*, *14*, 47-55. https://doi.org/10.1146/annurev.pa.14.040174.000403

Imanpoor, M. R., Bagheri, T., & Hedayati, S. A. A. (2010). The anesthetic effects of clove essence in Persian sturgeon, *Acipenser persicus*. *World Journal of Fish and Marine Sciences*, *2*(1), 29-36.

Kasai, M., Hososhima, S., & Yun-Fei, L. (2014). Menthol induces surgical anesthesia and rapid movement in fishes. *The Open Neuroscience Journal*, *8*, 1-8. https://doi.org/10.2174/1874082001408010001

Keifer, D., Ulbricht, C., Rae Abrams, T., Basch, E., Giese, N., Giles, M., DeFranco Kirkwood, C., Miranda, M., & Woods, J. (2007). Peppermint (*Mentha piperita*): An evidence-based systematic review by the natural standard research collaboration. *Journal of Herbal Pharmacotherapy*, *7*(2), 91-143. https://doi.org/10.1080/J157v07n0207

Mazandarani, M., & Hoseini, S. M. (2016). Menthol and 1, 8-cineole as new anesthetics in common carp, *Cyprinus carpio* (Linnaeus, 1758). *Aquaculture Research*, *48*, 3041-3051. https://doi.org/10.1111/are.13136

Mazandarani, M., & Hoseini, S. M. (2018). Anesthesia of juvenile Persian Sturgeon, *Acipenser persicus* (Borodin, 1897), by peppermint, *Mentha piperita*, extract – Anesthetic efficacy, stress response and behavior. *International Journal of Aquatic Biology*, *5*(6), 393-400. https://doi.org/10.22034/ijab.v5i6.391

McKay, D. L., & Blumberg, J. B. (2006). A review of the bioactivity and potential health benefits of

peppermint tea (*Mentha piperita* L.). *Phytotherapy Research, 20*(8), 619-633. https://doi.org/10.1002/ptr.1936

Moghim, M., Kor, D., Tavakolieshkalak, M., & Khoshghalb, M. B. (2006). Stock status of Persian Sturgeon (*Acipenser persicus* Borodin, 1897) along the Iranian coast of the Caspian Sea. *Journal of Applied Ichthyology, 22*, 99-107. https://doi.org/10.1111/j.1439-0426.2007.00935.x

Mohammadizarejabad, A., Bastami, K. D., Sudagar, M., & Motlagh, S. P. (2009). Hematology of great sturgeon (*Huso huso* Linnaeus, 1758) juvenile exposed to clove powder as an anesthetic. *Comparative Clinical Pathology, 19*, 465-468. https://doi.org/10.1007/s00580-009-0886-8

Ortuno, J., Esteban, M. A., & Meseguer, J. (2002). Effects of four anesthetics on the innate immune response of gilthead seabream (*Sparus aurata* L.). *Fish & Shellfish Immunology, 12*, 49-59. https://doi.org/10.1006/fsim.2001.0353

Padiyoor, B. A., Benno Pereira, F. G., & Jayaprakas, V. (2017). Assessment of clove oil and benzocaine anesthesia on hematological and histopathological profile of *Haludaria fasciata*. *Journal of Biodiversity and Environmental Sciences (JBES), 10*, 27-37. Retrieved from: https://innspub.net/assessment-of-clove-oil-and-benzocaine-anaesthesia-on-haematological-and-histopathological-profile-of-haludaria-fasciata/

Pedrazzani, A. S., & Neto, A. O. (2014). The anaesthetic effect of camphor (*Cinnamomum camphora*), clove (*Syzygium aromaticum*) and mint (*Mentha arvensis*) essential oils on clown anemonefish, *Amphiprion ocellaris* (Cuvier 1830). *Aquaculture Research, 47*, 769-776. https://doi.org/10.1111/are.12535

Pereira-da-Silva, E. M., de Oliveira, R. H. F., & Nero, B. D. (2014). Menthol as an anesthetic for lambari *Astyanax altiparanae* (Garutti & Britski 2000): Attenuation of stress responses. *Aquaculture Research, 47*, 1413-1420. https://doi.org/10.1111/are.12599

Pettersen, J. M., Bracke, M., Midtlyng, P. J., Folkedal, O., Stien, L. H., Steffenak, H., & Kristiansen, T. (2014). Salmon welfare index model 2.0: An extended model for overall welfare assessment of caged Atlantic salmon based on a review of selected welfare indicators and intended for fish health professionals. *Reviews In Aquaculture, 6*, 162-179. https://doi.org/10.1111/raq.12039

Pittler, M. H., & Ernst, E. (1998). Peppermint oil for irritable bowel syndrome: A critical review and meta-analysis. *The American Journal of Gastroenterology, 93*, 1131-1135. https://doi.org/10.1111/j.1572-0241.1998.00343.x

Posner, L. P., Scott, G. N., & Law, J. M. (2013). Repeated exposure of goldfish (*Carassius auratus*) to tricaine methanesulfonate (MS-222). *Journal of Zoo and Wildlife Medicine, 44*, 340-347. https://doi.org/10.1638/2012-0151R1.1

Roberts, R. J. (2012). *Fish pathology*. (4th edition). New Jersey, US: Wiley-Blackwell: Hoboken.

Sattari, M., Bibak, M., Bakhshalizadeh, S., & Forouhar Vajargah, M. (2020). Element accumulations in liver and kidney tissues of some bony fish species in the Southwest Caspian Sea. *Journal of Cell and Molecular Research, 12*(1), 33-40. https://doi.org/10.22067/jcmr.v12i1.85975

Saydmohammed, M., & Pal, A. K. (2009). Anesthetic effect of eugenol and menthol on handling stress in *Macrobrachium rosenbergii*. *Aquaculture, 298*, 162-167. https://doi.org/10.1016/j.aquaculture.2009.10.020

Stoskopf, M., & Posner, L. P. (2008). Anesthesia and analgesia in laboratory animals (Chapter 21). In: Fish, R., Brown, M., Danneman, P., & Karas, A. (Eds.) *American college of laboratory animal medicine* (pp. 519-534). New York City: Elsevier. https://doi.org/10.1016/B978-0-12-373898-1.X5001-3

Tarkhani, R., Imani, A., Jamali, H., & Ghafarifarsani, H. (2017). Anaesthetic efficacy of eugenol on various size classes of angelfish (*Pterophyllum scalare* Schultze, 1823). *Aquaculture Research, 48*(10), 5263-5270. https://doi.org/10.1111/are.13339

Teixeira, E. G., Moreira, A. G. L., Moreira, R. L., & Lima, F. D. S. (2011). Menthol as an anesthetic for different size classes of Nile tilapia science. *Archives of Veterinary Science, 16*, 75-83.

Vajargah, M. F., Imanpoor. M. R., Shabani, A., Hedayati, A. A., & Faggio, C. (2019). Effect of long-term exposure of silver nanoparticles on growth indices, hematological, biochemical parameters, and gonad histology of male goldfish (*Carassius auratus gibelio*). *Microscopy Research Technique, 82*(7), 1224-1230. https://doi.org/10.1002/jemt.23271

Vajargah, M. F., Mohsenpour, R., Yalsuyi, A. M., Galangash, M. M., & Faggio, C. (2021). Evaluation of the histopathological effect of roach (*Rutilus rutilus caspicus*) in exposure to sub-lethal concentrations of Abamectin. *Water, Air, & Soil Pollution, 232*(5), 1-8. https://doi.org/10.1007/s11270-021-05128-w

Velisek, J., Svobodova, Z., Piackova, V., Groch, L., & Nepejchalova, L. (2005). Effects of clove oil anesthesia on common carp (*Cyprinus carpio* L.). *Veterinarni Medicina, 50*, 269-275. https://doi.org/10.17221/5623-VETMED

Velisek, J., Wlasow, T., Gomulka, P., Svobodova, Z., & Novotny, L. (2007). Effects of 2-phenoxyethanol anesthesia on sheatfish (*Silurus glanis* L.). *Veterinarni Medicina, 52*(3), 103-110. https://doi.org/10.17221/2011-VETMED

Weber, R. A., Peleteiro, J. B., Martín, L. O. G., & Aldegunde, M. (2009). The efficacy of 2-phenoxyethanol, metomidate, clove oil, and MS-222 as anesthetic agents in the Senegalese sole (*Solea senegalensis* Kaup 1858). *Aquaculture, 288*, 147-150. https://doi.org/10.1016/j.aquaculture.2008.11.024

Wedemeyer, G. A., Barton, B. A., & McLeay, D. J. (1990). Stress and acclimation. In: Schreck, C. B., Moyle, P.B. (Eds.) *Methods for fish biology*. (pp. 451-488), Bethesda, US: American Fisheries Society.

Yalsuyi, A. M., Hajimoradloo, A., Ghorbani, R., Jafari, V. A., Prokić, M. D., & Faggio, C. (2021a).

Behavior evaluation of Rainbow trout (*Oncorhynchus mykiss*) following temperature and ammonia alterations. *Environmental Toxicology and Pharmacology*, *86*, 103648. https://doi.org/10.1016/j.etap.2021.103648103648

Yalsuyi, A. M., Vajargah, M. F., Hajimoradloo, A., Galangash, M. M., Prokić, M. D., & Faggio, C. (2021b). Evaluation of behavioral changes and tissue damages in common carp (*Cyprinus carpio*) after exposure to the herbicide glyphosate. *Veterinary Sciences*, *8*(10), 218. https://doi.org/10.3390/vetsci8100218

Yousefi, M., Hoseini, S. M., Vatnikov, Y. A., Nikishov, A. A., & Kulikov, E. V. (2018). Thymol as a new anesthetic in common carp (*Cyprinus carpio*): Efficacy and physiological effects in comparison with eugenol. *Aquaculture*, *495*, 376-383. https://doi.org/10.1016/j.aquaculture.2018.06.022