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WILD MINT, *Mentha piperita* TO ANESTHETIZE FINGERLINGS OF THE COMMON CARP *Cyprinus carpio*

Samer S. Alshkarchy[1], Khalidah S. Al-Niaeem[2] and Amjed K. Resen[2]

[1]*Department of Pathology and Poultry Disease, College of Veterinary Medicine, Al-Qasim Green University, Iraq*
[2]*Department of fisheries & marine resources, College of Agriculture, University of Basrah, Iraq*

Abstract

Extracted solution of Wild mint (*Mentha piperita*) with six concentrations (100, 150, 200, 250, 300, and 350 mg/L) was used to anesthetize common carp *Cyprinus carpio* fingerling (total length 8.55 ± 0.41 cm and total weight 7.75±1.18 gm). Fish behavior was noticed during anesthesia. Results showed that extracted solution of wild mint has partial and overall anesthesia effect on *Cyprinus carpio* fingerling with the inverse relationship between the concentrations and the time needed to reach partial and overall anesthesia, and a direct relationship between concentrations and time needed for fish recovery. The best results were obtained by using a concentration of 350 mg/l, where the time for partial anesthesia was 8.15 ±0.64 min time for overall anesthesia was 10.15±0.30 min. The time needed for partial recovery was 16.25±1.24 min and the time needed for overall recovery was 21.2±1.11 min. Fish behavior observations revealed a difference ranging from slow swimming with increasing breathing movements to vertical swimming near the surface, then laying at the bottom, and too much decrease in breathing movements. Results appeared that there were no significant differences ($p \geq 0.01$) between glucose concentration in fish blood plasma after recovery and control fishes, so it was concluded that these fishes exhibited no stress during anesthesia by using the wild mint extracted solution. The results showed that there were no significant differences ($p \geq 0.01$) in both ALP, GOT, GPT, CK, and LDH enzymes among fishes after recovery in comparison with the control fishes. This indicated that the treated fishes exhibited no physical damage that might lead to poor health conditions. In addition, the results showed that there were significant differences ($p \leq 0.01$) in blood parameters (RBC and Hb) among fishes after one week from the end of exposure to an extracted solution of wild mint in comparison with the control fishes.

Key words: *Mentha piperita*, Anesthesia, *Cyprinus carpio* and Fingerling.

1. Introduction

Anesthesia is used in the commercial fish sector and in scientific studies for the purpose of preventing injuries and reducing stress in fish. Currently, an increasing number of anesthetic agents are used in aquaculture(Alshkarchy *et al*., 2020).

Some narcotic substances for fish have a synthetic origin, such as MS-222, benzocaine and 2-phenoxyethanol (Priborsky and Velisek, 2018). Others are natural products, such as essential oils of vegetable origin (Hoseini *et al*., 2019). It is considered an alternative to synthetic preparations (Readman *et al*., 2013), mainly due to its low price, availability, efficiency, environmental friendliness, and safe for fish and humans.

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The characteristics mentioned by Coyle *et al*. (2004). The anesthetics used to anesthetize fish, namely: the use of low concentrations of anesthesia, rapid results in anesthesia and recovery, soluble in water and alcohol, etc.

Mentha piperita oil is another essential oil that has an anesthetic effect on fish and is a natural product that has an antiseptic, analgesic, and bactericidal effect (Tsuchiya, 2017). *Mentha piperita* is native to Europe and the Middle East but is cultivated all over the world. The essential oil of this plant is mainly rich in menthol and cineole (De Oliveira Hashimoto *et al*., 2016; Rohloff, 1999). De Oliveira Hashimoto *et al*. (2016) confirmed the activity of the drug-doped *Mentha piperita* in Nile tilapia (*Oreochromis niloticus*). Extracts of *Mentha piperita* by Hydro-distillation method. It contains menthol (27.5 %), menthofuran (22.5 %), pulegone (12.8 %), menthyl acetate (12.5 %) and menthone (11.0%) at concentrations of 40 - 160 $\mu L/L^{-1}$ anesthetized fish during 1200 - 300 sec. In addition, antiparasitic activity against monogenean worms (De Oliveira Hashimoto *et al*., 2016). Native to Europe and the Middle East cultivated in other countries and regions (Hoseini *et al*., 2019).

The efficacy of peppermint oil and its main component (menthol) as an anesthetic has been tested in a variety of mostly marine fish, such as the clown fish *Amphipriono cellaris* (Pedrazzani and Neto, 2016), Nile tilapia *Oreochromis niloticu* (Rezende *et al*., 2017). American trout *Oncorhynchus mykiss* (Metin *et al*., 2015), Persian sturgeon *Acipenser persicus* (Mazandarani and Hoseini, 2017), silverfish *Rhamdia quelen* (Dos Santos *et al*., 2017), Silverfish African catfish *Clarias gariepinus* (Popoola *et al*., 2015)

Common carp is one of the most famous fish in Iraq. Much research has been done on it, for example. Extraction of eggs or sperm in IVF and for research purposes, morphological studies, hormonal injections, vaccination, and transfer. All of these applications cause stress to fish, which in turn negatively affects fish behavior and fish physiology (Ross and Ross, 2009). There is no information, in the scientific literature, on the anesthetic effect of peppermint oil on common carp, regardless of its age stage of development, which determined its use in this study. The study aims to prove the efficacy of an anesthetic: peppermint oil, in different concentrations and the time required for induction and recovery from anesthesia in fingerlings common carp.

2. Materials and Methods

Common carp were brought from the ponds of the Marine Science Center - University of Basra (average total length 8.55 ± 0.41 cm and average total weight 7.75 ± 1.18 g). The fish were placed in the laboratory for 14 days to adapt to glass aquariums of dimensions (30 x 30 x 50) cm (50 fish and 10 other fish as stock in case of fatalities in the anesthesia experiments). The water temperature was (22.66 ± 0.57) 0 °C and the pH was (7.8 ± 0.05). An appropriate amount of catnip leaves was taken, and grinding well using an electric grinder, then prepared the required concentrations in the experiment (100, 150, 200, 250, 300, and 350 mg/L) after dissolving them in hot water, leaving them for an hour to extract the total active substances, then filtering them to get rid of suspended materials (Twaij *et al*., 1983) and modified by Al-Niaeem (2006). Then other glass aquariums for anesthesia experiments, the dimensions of which are (24 x 14 x 10) cm. 10 fish in each concentration (Three replicates for each concentration) were used to perform the anesthesia experiments with the fish behavior monitored to reach the partial stress state in addition to reaching the total stress state, as well as the fish until the partial and total recovery state. The concentration of glucose in the blood plasma was measured using a laboratory kit ready-made by the French company Bio Maghreb with a spectrophotometer at a wavelength of 505 nm.

The activity of liver enzymes was estimated by means of a ready-made diagnostic kit (kit) and by using a spectrophotometer. The activity of the enzyme alkaline phosphatase (ALP) was estimated at a wavelength of 510 nm. The activity of Glutamic Oxaloacetic

Transaminase (GOT) was estimated at a wavelength of 546 nm and that of Glutamic Pyruvic Transaminase (GPT) at a wavelength of 546 nm. The activity of the enzyme (CK) Creatine Kinase at a wavelength of 560 nm and that of the enzyme (LDH) were estimated. Lactate Dehydrogenase

The following tests were done on fish blood: Hemoglobin (Hb), red blood cell count (RBC), and WBC count. The ready-made statistical program was used in the statistical analysis SPSS Statistics V. 20 to test the differences between the means for all the tests and the use of the least significant difference test R.L.S.D. To determine those differences at the level of significance 0.01.

3. Results

All concentrations used for partial and total anesthesia on fish showed a variation in the time of obtaining partial and total anesthesia and partial and total recovery. The concentration of 350 mg/L recorded the best time for Overall anesthesia and Overall recovery. The average time for partial anesthesia ranged from 8.15±0.64 minutes, and the average time for total anesthesia was 10.13± 0.30 minutes, while the concentration of 100 mg/L was characterized by time to reach partial and total anesthesia 40.5±0.52 and 66.2±1.22 minutes, respectively, but the total recovery period was the shortest among the concentrations used 7.36±0.41 mins. Significant differences ($p \leq 0.01$) were found in the partial and total anesthesia and the partial and total recovery of common carp among the six drug concentrations (Table - 1) shows the inverse relationship between the concentration used and the time to reach the total partial anesthesia, while the relationship was direct between the concentration used and the time to reach the state of total recovery for carp fish.

Behavioral observations

Behavioral observations of carp fish showed a rapid increase in respiratory movements from time to time after five minutes of anesthesia. Partial anesthesia occurred with rapid movements and in different directions and escape when trying to catch it, an increase in the number of movements of operculum significantly and then begin to decrease, then a decrease in the movement of the caudal fin, and total anesthesia occurred with the stability of the fish on the bottom of aquarium and simple positional movements and ease of grasping. The fish are placed at the bottom of the tank in a lateral position, a very large decrease in the movement of the gill cap, and then a final stop in swimming and the movement of the caudal fin stops. Total recovery was achieved by movement of the last part of the anesthesia, which is the caudal fin, followed by an increase in the movement of the operculum, in addition to very slow swimming at first, then normal swimming.

Glucose in Fish Blood Plasma

The current study showed that there were no significant differences ($p \geq 0.01$) for the concentration of glucose in the blood plasma of fish after the state of total recovery after comparing with the control fish (Table - 2).

Efficacy of liver enzymes and fish blood parameters

The current study showed that there were no significant differences ($p \geq 0.01$) for the ALP, GPT, GOT, CK and LDH enzymes level in the serum of fish after total recovery from exposed to the aqueous extract of *Mentha piperita* compared to control fish (Table - 3).

Table - 1: Times of partial and total anesthesia and partial and total recovery when using different dose of *Mentha piperita* aqueous extract to anesthetize of *Cyprinus carpio* fingerlings

Concentration (mg/ l)	Partial anesthesia time (min.)	Overall recovery time (min.)	Partial recovery time (min.)	Overall anesthesia time (min.)
100	0.52 ± 40.5 a	66.2 ± 1.22 a	3.24 ± 1.30 a	7.36 ± 0.41 a
150	0.82 ± 36.3 b	53.5 ± 1.27 b	5.2 ± 1.14 b	10.73 ± 0.48 b
200	0.64 ± 28.5 c	41.3 ± 1.25 c	6.46 ± 1.22 c	13.43 ± 0.45 c
250	19.51 ± 0.42 d	34.37 ± 0.65 d	10.01 ± 2.12 d	16.65 ± 1.25 d
300	15.47 ± 0.62 e	28.51 ± 0.45 e	12.13 ± 1.40 e	18.56 ± 1.23 e
350	8.15 ± 0.64 f	10.13 ± 0.30 f	16.25 ± 1.24 f	21.2 ± 1.11 f

*Means ± SD
*Means with the same letter in the same column are not significantly different

Table - 2: Blood Plasma Glucose of *Cyprinus carpio* Fingerling after Total recovery form Anesthesia by Aqueous extract of *Mentha piperita*

Treatments	Control	100	150	200	250	300
Glucose (mg/100 ml)	60.67±4.08a	62.41±3.10a	60.53±2.17a	61.80±2.20a	64.01±2.01a	67.03±2.24a

*Means ± SD
*Means with the same letter in the same raw are not significantly different

Table - 3: Effect of different concentrations of aqueous extract of *Mentha piperita* on (ALP, GOT GPT, CK and LDH) *Cyprinus carpio* fish after total recovery

Parameter (u/l)	Control	100	150	200	250	300	350
ALP	53.24±2.12 a	57.35±2.03 a	55.32±2.10 a	57.51±1.1 a	57.24±2.13 a	53.52±2.11 a	56.42±2.20 A
GOT	44.15±0.17 a	37.24±2.22 a	38.21±1.21 a	42.24±0.25a	35.16±2.18 a	36.20±2.16 a	30.24±2.12 A
GPT	4.33±0.20 a	4.30 ±1.17 a	4.33±0.45 a	4.24±0.25 a	4.23±1.20 a	4.23±0.23 a	5.32 ±0.21 A
CK	38.47±2.17 a	36.43±2.20 a	36.14±2.01 a	33.02±3.35 a	33.17±2.10 a	38.21±2.21 a	33.54±1.28 A
LDH	75.26±1.25 a	72.21±1.32 a	75.21±1.02 a	74.20±1.20 a	68.04±2.55 a	67.26±2.14 a	74.13±1.22 A

* Means ± SD
*Means with the same letter in the same raw are not significantly different

4. Discussion

Anesthesia fish with essential oils can provide many health benefits such as antioxidant and antimicrobial effects (Chaieb *et al.*, 2007; Gressler *et al.*, 2014). *Mentha piperita* aqueous extract has often few studied as an anesthetic in fish, and in this regard there are no data for its application in Common carp fingerlings. The results of the follow-up of the behavior of carp fish in the catnip anesthetic solution showed that the fish went through three stages when anesthetized, which are i) The fish activity decreased; ii) The respiratory rate increased, iii) The appearance of signs of loss of balance with the start of vertical swimming and the decrease in respiratory movements, and finally the turn to the side and the decrease in Respiratory movements, which may be due to the sensation of a chemical compound by the olfactory and gustatory systems. More severe reactions to exposure to peppermint extract may be due to activation of cold nociceptors (Wei and Seid, 1983; Kasai *et al.*, 2014; Mazandarani and Hoseini, 2017). Also, the behavioral response in the second stage of anesthesia (operculum closure) appears to be related to the activation of gill cold nociceptors, forcing the fish to block the gill exposure to the Mintha extract (Kasai *et al*., 2014; Mazandarani and Hoseini, 2017) reported rapid movements in fish anesthetized with menthol, which could be due to activation of cold nociceptor activation receptors. The (350 mg/L) of aqueous extract of *Mentha piperita* recorded the best time for Overall anesthesia and Overall recovery. The average time for partial anesthesia ranged from 8.15±0.64 minutes, and the average time for Overall anesthesia was 10.13± 0.30 minutes the reason that the agitation time obtained in this study is higher compared to the previous studies may be due to the higher fish weight and lower water temperature in this study compared to the previous studies. An increase in fish weight affects the induction time. For example, Hoseini *et al*. (2015) found a decrease, (Stehly and Gingerich, 1999) found no change in induction time due to increased fish weight. Park *et al*. (2009) showed that high temperature significantly reduces the induction and recovery time of clove oil in the rock bream, *Oplegnathus fasciatus*. There is little information about the induction and recovery time of mint extract in fish. Façanha and Gomes (2005) found an induction time of 105 - 358 seconds for 250 - 50 mg L^{-1} menthol in *Tambaqui*, *Colossoma macropomum*, which is lower than this study. The reason for the difference between the results of the current study and those by Façanha and Gomes (2005) and Kasai *et al*. (2014) not exactly shown. In addition to environmental factors that affect anesthesia efficacy. There are several studies showing an anesthetic stress response in fish (Aupérin *et al*., 1997; Holloway *et al*., 2004; Hoseini *et al*., 2011; Park *et al*., 2009). Glucose is the best indicator of stress in fish. In general, the present results showed that the aqueous extract of *Mentha piperita* did not show a difference in glucose levels in *Cyprinus carpio* fingerlings after total recovery when compared to the control treatment. Time is a functional indicator of several factors, including stress, and therefore glucose in the blood plasma is an important factor (Martínez-Porchas *et al*., 2009). Also, the concentration of fish liver enzymes (ALP, GOT, GPT, CK and LDH) did not differ in the current study after the total recovery of fish when compared to the control treatment, in addition to it confirming that the use of aqueous extract of *Mentha piperita* did not affect the health of fingerlings of *Cyprinus carpio* fish. Its use did not affect an increase or decrease in the secretion of liver enzymes. There is no study that monitors serum glucose in fish anesthetized with aqueous extract of *Mentha piperita*, and the results agree with several researchers on different types of fish and the use of cloves as an anesthetic for fish, as Min Ouk Park *et al*. (2008) showed no change in Blood in grouper fish, *Epinephelus bruneus* after 1 - 6 hours when anesthetized with clove oil but significantly increased after 12 - 48 hours. On the other hand, Park *et al*. (2009) found a significant increase in serum cortisol in the rock bream, *O. fasciatus* 0 - 12 hrs after anesthesia with clove oil, which returned to normal 24 - 72 hours after anesthesia. However, further studies are necessary to find out the reason for these findings.

4. Conclusions and Recommendations

We conclude from the current study that aqueous extract of *Mentha piperita* aqueous can be used for anesthesia in *Cyprinus carpio*, yet it is not as suitable as clove oil, because higher stress induction. *Cyprinus carpio* Fingerlings.

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