

THE EFFICACY OF MYRTLE (*Myrtus communis* L.) AS AN ANAESTHETIC ON COMMON CARP *Cyprinus carpio* JUVENILES

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ABSTRACT

Extracted solution of myrtle (*Myrtus communis*) with five concentrations (400, 500, 600, 700 and 800 mg/l) were used to anesthetize juveniles of the common carp *Cyprinus carpio* (total length 8.91 ± 0.31 cm and total weight 7.72 ± 1.19 gm) instead of the traditional use of MS-222. Fish behavior was noticed during anesthesia. MS-222 is an expensive chemical substance that causes cancer for workers and fish consumers and also pollutes the aquatic environments. Results showed that extracted solution of myrtle have partial and overall anesthesia effect on juvenile common carp with inverse relationship between the concentrations and the time needed to reach partial and overall anesthesia, and also direct relationship between concentrations and time needed for fish recovery. Best results were obtained by using a concentration of 700 mg/l, where time for partial anesthesia was 10 ± 1.22 min., time for overall anesthesia was 15 ± 1.34 min., time needed for partial recovery was 18 ± 2.32 min. and time needed for overall recovery was 22 ± 2.51 min. Fish behavior observations revealed a difference ranging from slow swimming with increasing in breathing movements to vertical swimming near the surface, then laying at bottom and too much decrease in breathing movements. Results appeared that there were no significant differences ($p > 0.01$) 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 myrtle extracted solution. The results showed that there were no significant differences ($p > 0.01$) in both alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactic dehydrogenase (LDH) and creatine kinase (CK) (serum enzymes) among fishes after recovery in comparison with the control fishes. This indicated that the treated fishes exhibited no physiological effect which might lead to poor health condition.

KEYWORDS: Anaesthetic, recovery, myrtle, serum enzymes, common carp.

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INTRODUCTION

During aquaculture and stocking activities, fishes are faced with several potential stressors, in particular, transportation, capture and handling procedures, a highly crowded and confined farming environment, possible air exposure and variation in water quality are all factors that may increase the stress level of organisms (Weinert *et al.*, 2015) and have significant effects on fish physiology and survival (Lepic *et al.*, 2014).

Over the years, a large number of chemicals and agents have been applied to fish in the hopes of inducing anesthesia, with varying degrees of success, however, only a few of them have found in a widespread usage sufficient to allow even a basic understanding of their effects and optimal application (Aliakbar and Hadideh,

2015). Many fish anesthetics reported in published compilations have been used only sporadically and usually without the adjunct assessment of key physiologic impacts of the drug, for the most part, those drugs are no longer employed in fish anesthesia, although they do seem to be rediscovered on occasion (Ghanawi *et al.*, 2013). It is often advisable to identify the lowest effective doses of different anesthetics in a specified species, as the responses to the same anesthetic may vary considerably among different species (Zargham *et al.*, 2013).

Tricaine methanesulfonate, Benzocaine, Phenoxyethanol, Isoeugenol, Quinaldine, Propofol, Clove (Carnation Buds) as anesthesia, tobacco, *Zataria multiflora*, *Nicotiana tabacum* and *Myrsitca fragrans* are all used as fish anesthetics to some degree and with different activity (Zahl *et al.*, 2012; Ghanawi *et al.*, 2013;

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Zargham *et al.*, 2013; Aliakbar and Hadideh, 2015; Santos *et al.*, 2015; Baiju *et al.*, 2017; Al-Niaeem *et al.*, 2017a; Al-Niaeem *et al.*, 2017b and Al-Niaeem *et al.*, 2019).

There are no studies on anaesthetic effect of myrtle, *Myrtus communis*, and there is no information on their effects on fish physiology. Therefore, in this study, it was designed to determine anesthetic and some blood serum enzymes of myrtle on common carp juveniles, *Cyprinus carpio*.

MATERIALS AND METHODS

The experiment was conducted on common carp juveniles (*C. carpio*) in which their weights was (7.53 ± 1.22 g), which were brought from the fish farm of Marine Science Center, University of Basrah (using 25 common carp, December 2018). They were pre-acclimated to laboratory conditions and fed with commercial pellets prior to the feeding trials. In this experiment the anesthetics effect of myrtle extract was investigated at five concentrations and control, for each concentration three replicate were used, anesthetics were dosed as followed: 400, 500, 600, 700 and 800 mg/l were prepared according to Al-Niaeem (2006); Al-Niaeem and Al-Yassein (2009). Three replicates in each concentration were used. Each replicate aquarium was stocked with three fish.

The aquaria (replicates) were randomly allocated to minimize the differences among treatments. The continuous water flow discharged non-consumed feed and feces particles from the aquaria. Water temperature in aquariums during the experimental trials was $17.53 \pm 0.42^{\circ}$ C and pH was 8.1 ± 0.03 .

Studied characteristics

- 1- Times of anesthesia and recovery according to anesthesia and recovery stages :Anesthesia time and recovery time were measured to the nearest minutes (Brown, 2011).
- 2- Fish behavior during anesthesia and recovery time.
- 3- Blood serum enzymes that measured were aspartate aminotransferase (AST), alanine

aminotransferase (ALT), alkaline phosphatase (ALP), creatine kinase (CK) and lactic dehydrogenase (LDH).

The blood samples were collected by suction of the caudal peduncle blood vessel from each fish of different groups. Blood was then separated by centrifugation (1500 rpm) for three minutes (Yang and Chen, 2003). The levels of serum enzymes were assayed according to the instructions provided with the corresponding enzymatic kits (Randox Company). The statistical calculations of the results were completed using SPSS version 20, one way (ANOVA) to determine the difference between the means.

RESULTS

All fish used in the present study were healthy morphologically as indicated by their activity and external appearance. No mortality was observed during the acclimatization period. Furthermore, no deaths or other adverse effects were occurred within 48 h following recovery from anesthesia for the short term and long term periods of mortality. Blood sampling was performed while the fish were still deeply anaesthetized. All the fish exposed to different treatments recovered well and returned to normal behavior with response to feeding, surfacing activity, swimming and respond to external stimuli after the anesthetic treatment.

Blood sampling was performed while the fish were still deeply anaesthetized.

Table (1) shows the induction time of *C. carpio* which decreased with increasing concentrations of myrtle, (*M. communis*). At 700 mg/l (the best concentrations), the time to reach a complete anaesthesia was 15 min. which is significantly different ($P < 0.01$) compared with control group.

There was a clear direct relationship between recovery time and concentrations of the anaesthetic. The longest recovery time was 45 ± 2.12 min. at a concentration of 800 mg/l, while the shorter recovery time was 7 ± 1.25 min. at a concentration of 400 mg/l myrtle.

Table (1): Effect of myrtle concentrations on anesthesia and recovery time in common carp.

Concentration (mg/l)	Partial anaesthesia time (min.)	Overall anaesthesia time (min.)	Partial recovery time (min.)	Overall recovery time (min.)
Mean ± SD*				
400	64 ± 2.14 ^a	85 ± 5.13 ^a	5 ± 1.15 ^a	7 ± 1.25 ^a
500	48 ± 1.20 ^b	52 ± 5.50 ^b	8 ± 1.50 ^b	10 ± 2.15 ^b
600	22 ± 1.10 ^c	33 ± 2.64 ^c	16 ± 2.52 ^c	18 ± 2.42 ^c
700	10 ± 1.22 ^d	15 ± 1.34 ^d	18 ± 2.32 ^d	22 ± 2.51 ^d
800	8 ± 1.43 ^e	10 ± 1.21 ^e	30 ± 2.33 ^e	45 ± 2.12 ^e

*Different letters in the same column are significantly different (P<0.01).

The biochemical indices (enzymes) measured in the blood serum of the fish (AST, ALT, ALP, CK and LDH) is presented in Figure (1). All the anaesthetized groups at all the stages of anesthesia and recovery had no significant differences (P>0.01) between these biochemical indices for anaesthetic fishes compared with control group, except myrtle concentrations 800 mg/l, the stages of anesthesia and recovery had

significant differences (P<0.01) between these biochemical indices (CK and LDH) for anaesthetic fishes compare with control treatments, their values are very high, which is an unhealthy condition for the liver. Figure (2 & 3) shows the blood serum enzymes of common carp for 7 and 14 days after the completion of the study (no significant differences (P>0.01).

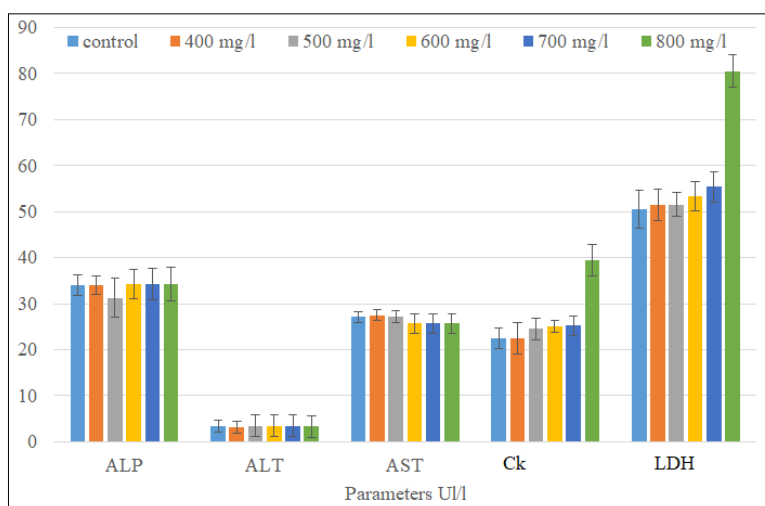


Fig. (1): Effect of myrtle concentrations on blood serum enzymes of common carp anaesthesia.

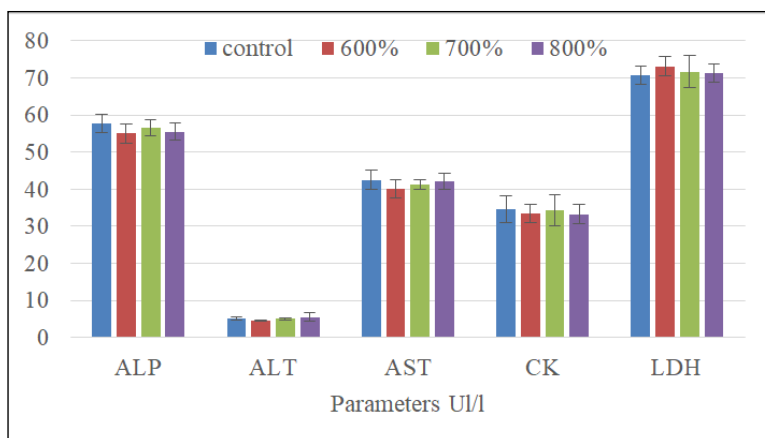


Fig. (2): The blood serum enzymes of common carp for 7 days after the completion of the study.

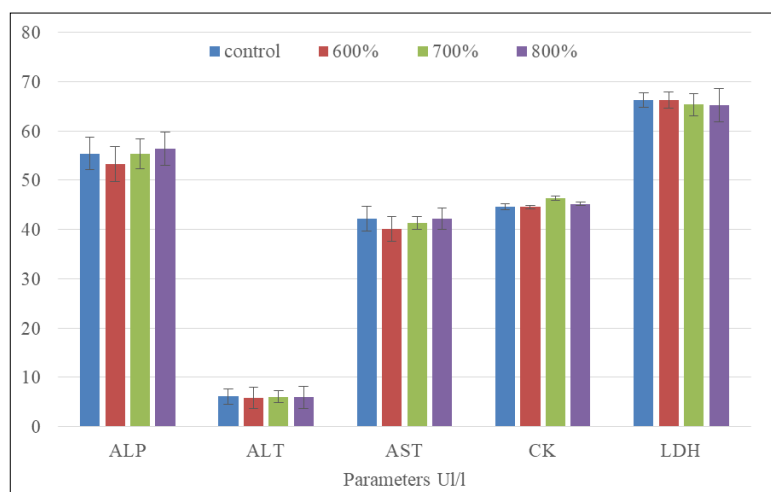


Fig. (3): The blood serum enzymes of common carp for 14 days after the completion of the study.

DISCUSSION

Myrtus communis, the common myrtle, is a species of flowering plant in the myrtle family Myrtaceae. It is an evergreen shrub native to southern Europe, North Africa, western Asia, Macaronesia and the Indian Subcontinent (Marinho *et al.*, 2009). It was used as a sedative in cases of epilepsy, and removal of pain in the lower abdomen, and also described for the treatment of respiratory diseases, such as respiratory tract infections; it is a calming cough and a dwelling of chest pain, the essential oils are active ingredients of myrtle (Birhanie, 2016).

The results of the present study showed that anesthesia time decreased with increasing concentrations of myrtle plant, and there were significant differences ($P < 0.01$) in anesthesia time between different concentrations (400, 500, 600, 700 and 800 mg/ l). Also, the results revealed direct relationship between recovery time and concentrations of the anesthetic, with significant differences ($P < 0.01$). When using anesthetics, it is expected that there will be a strong negative correlation between the applied concentration and the time required to induce anaesthesia to the desired stage, as observed previously for several fish species (Pawar, 2001; Feng *et al.*, 2011; Mortazevi *et al.*, 2012 and Kamble *et al.*, 2014). Long exposure to anaesthetic led to more anaesthetic absorption by fish which, in turn, lengthened the recovery time. The time for total loss of reflex decreased as the concentration of myrtle increased. This is

in good accord with the previous studies that suggested that the total loss of reflex decreased with increasing anesthetic concentration (Pawar, 2001 and Kamble *et al.*, 2014). The state of anesthesia induced by anesthetic concentration may vary among the species, and within the same one, it does pursuant to the size (Mortazevi *et al.*, 2012).

On the other hand, Kamble *et al.* (2014) pointed out that long exposure to anesthetic led to more anesthetic absorption by fish which, in turn, lengthened the recovery time. The statement is not completely trustworthy otherwise this could be proved with present result because if it could be said that longer exposure to low concentration of the anesthetic leads to more anesthetic absorption. It could be said that short exposure to high anesthetic concentration do it as well. On the other hand, (Weyl *et al.*, 1996) pointed out that compared with anesthesia duration, anesthetic concentration plays more important role on the recovery time. It is believed that the independence of the recovery time from the anesthesia duration, as a result of that anesthetic, is taken up by the fish through a concentration gradient at the gill interface. Therefore, when equilibrium level established between the gill and anesthetic solution, no further anesthetic will taken up by the fish, and during recovery, the anesthetic agent is leaked through such gradient. Therefore, the recovery time is controlled by the anesthetic concentration but not duration of anesthesia (Weber *et al.*, 2009).

The blood is fluid connective tissue which acts as the main transporting system of the body in all animals. In poikilotherms like fish it plays vital role during every movement for maintaining the physiological nature of the body with respect to the fluctuating environmental parameters. Hematological parameters can provide needed information on the physiological status of fishes, and help the aquaculture and research personnel to make proper decisions to increase the survival of fishes. Results of current study indicated that the 85 min., 52 min., 33 and 15 min. exposure to myrtle at different concentrations had no significant differences ($P>0.01$) on the biochemical indices (ALP, AST, ALP, LDH and CK) measured immediately after anaesthesia. Also, there were no significant differences ($P>0.01$) between these biochemical indices for anaesthesia fishes compared with control group. Biochemical indices of blood serum can provide important information about the internal environment of the organism (Sindhu and Ramachandran, 2013; Bahrekazemi and Yousefi, 2017; Al-Niaeem *et al.*, 2017a, b; Al-Niaeem *et al.*, 2019). Values determined in the present study suggest that internal physiological rolls of organs and tissues of carp are no altered by myrtle anaesthesia, except myrtle concentrations 800 mg/l.

Anesthetics should be tested empirically for safety and efficacy in species or life stages of fish with little prior history of anesthesia (Matsche, 2011). Concentrations selected for induction and maintenance of anesthesia should achieve the desired anesthetic state necessary to accomplish procedures with minimal handling time and stressful side effects when possible (Dziaman, 2005).

Fish were observed for one week after the completion of the study. No fish died during that period and fish show normal activity indicating that this plant extract have no adverse effects. However, how this plant extracts affect the physiological processes are no clear. Therefore, further experiments need to be conducted to establish such effect, if any, if fish are to be anaesthetized using these extracts, for physiological experiments.

The study demonstrated that myrtle can be used as an effective anaesthetic for fishes (700 mg/l). Moreover, as an advantage, it refers to natural substance which has no any side effects on fishes and does not represent any hygienic

risks, instead of the traditional use of MS-222. MS-222 is an expensive chemical substance that causes cancer for workers and fish consumers and also pollutes the aquatic environments. However, further studies should be carried out to evaluate the metabolic effects of myrtle, and additional data are necessary in order to assess the effects on food intake, appetite and growth. We do not recommend using myrtle concentrations 800 mg/l, because the high values of CK and LDH are an unhealthy condition for the liver.

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بكارئینانا ئاڤا رووكئ ئاس (*Myrtus communis* L.)
دبھوشكرنا ماسییت كارب یی به ربه لاف *Cyprinus carpio*

پوخته

پینچ تیراتیین ژیکجودا (400 و 500 و 600 و 700 و 800 ملگم/ لتر) ژئاڤا رووهكئ ئاسئ هاتنه بكارئینان وهك پیکهورئ که رهستی MS-222 ژبو دبھوشكرنا ماسییت كارب (تیکرایئ درژاھیی هه میشهیی $0,31 \pm 8,91$ سم و تیکرایئ کیشه یا هه میشهیی $1,19 \pm 7,72$ غم زیده باری دویفچوونا رهفتارا وان ددهمئ دبھوشبوونئ دا. نه نجامان دیارکر کو ژئھاتئ ئاڤئ یئ ئاسئ یئ دبھوشکرهكئ بهشی بوو وهه میشهیی بوو ل سهر ماسیا و په یوه ندیا بهرو قازئ بوو دناقبه را تیراتیا هاتیه بكارئینان ودهمئ گه هشتبوونا بیهیشبوونا بهشی وهه میشهیی. ژلایهكئ دی قه، په یوه ندی یا تهردی بوو دناقبه را تیراتیئ ودهمئ گه هشتبوونا هشیاربوونا هه میشهیی یا ماسیا. باشتیرین دهرئه نجام هاتنه بدهستقه ئینان ددهمئ بكارئینانا 700 ملگم/ لتر ددهمئ تیکرایئ ددهمئ دبھوشبوونئ $1,22 \pm 10$ خولهك بوون. تیکرایئ ددهمئ دبھوشبوونئ یئ هه میشهیی گه هشته ± 15 1,34 خولهك. دیسان دویفچوونا رهفتارئ یا ماسیا جیاوازیهكا بهرچاڤ نیشا دا ژخیراییهكا نزم دمهله قانیئ دا دگهل زیده بوونهكا بهله دهه ناسئ دا زدهمهکی بو دمهكئ دپتر بو مهله قانیئ نیزیکی سهری تاكو ماسی بگه هیته ژیریا ئاڤئ، دگهل ل سهرخوویا هه ناسئ. نه نجامان دیسان دیارکر ب هه بوونا جیاوازیئ بهرچاڤ ($p > 0.01$) دچالاکئ یا انزیمی فوسفاتیزئ بنهکی (ALT) وانزیمئ قه گوھیزئ نه سپارتیز (AST) و قه گوھیزئ نه نین (ALT)، وانزیمئ لاکتیک دیهایدرئز (LDH) وانزیمئ کریاتین کاینیتیز (CK) دماسییت پشته هشیاربوونئ دبهراوهردی دگهل کونترولی وئه قه نیشانیئ نه بوونا کارتیکرئین فیزیولوجی ئه ویت دبنه ئه گهرئ لاوازبوونا بار و دوخین ساخله می.

استخدام مستخلص نبات الآس (*Myrtus communis* L.)
في تخدير اصبعيات أسماك الكارب الشائع *Cyprinus carpio*

الخلاصة

استعملت خمسة تراكيز (400 و500 و600 و700 و800) ملغم/ لتر من المستخلص المائي لنبات الآس *Myrtus communis* كبديل لمادة MS-222 لتخدير اصبعيات أسماك الكارب الشائع *Cyprinus carpio* (معدل الطول الكلي 8.91 ± 0.31 سم ومعدل الوزن الكلي 7.72 ± 1.19 غم)، إضافة إلى مشاهدة سلوكها خلال التخدير. بينت النتائج أن للمستخلص المائي للآس تخديراً جزئياً وكلياً على هذه الاسماك وكانت العلاقة عكسية بين التركيز المستخدم ووقت الوصول إلى التخديرين الجزئي والكلي، في حين كانت العلاقة طردية ما بين التركيز المستعمل ووقت الوصول إلى الإفاقة الكلية للأسماك. إن أفضل النتائج تحققت باستعمال التركيز 700 ملغم/ لتر، إذ كان معدل وقت التخدير الجزئي 10 ± 1.22 دقيقة، وبلغ معدل وقت التخدير الكلي 22 ± 2.51 دقيقة، أما معدل وقت الإفاقة الجزئي فكان 18 ± 2.32 دقيقة، وبلغ معدل وقت الإفاقة الكلي 15 ± 1.34 دقيقة. أظهرت المشاهدات السلوكية للأسماك تبايناً تراوح من بطئ في السباحة مع زيادة سريعة للحركات التنفسية بين فترة وأخرى إلى سباحة السمكة قريباً من السطح وصولاً إلى بقاء السمكة على القاع، فضلاً عن بطئ في التنفس. بينت النتائج عدم وجود اختلافات معنوية ($p > 0.01$) في فعالية أنزيم الفوسفاتيز القاعدي (ALP) وأنزيم ناقل الأستاتيز (AST) وناقل الألبومين (ALT) وأنزيم اللاكتيك ديهيدروجينيز (LDH) وأنزيم الكرياتينين كابينيز (CK) في الأسماك بعد الإفاقة الكلية مقارنة بأسماك السيطرة، وهذا يدل على عدم وجود تأثيرات فسلجية تؤدي إلى ضعف حالتها الصحية.