Open Access
2020, 17(1):42-47

Baghdad Science Journal

P-ISSN: 2078-8665
E-ISSN: 2411-7986

DOI: http://dx.doi.org/10.21123/bsj.2020.17.1.0042

The Efficacy of Poppy, *Papaver nudicaule* extract as an Anesthetic for the Common Carp, *Cyprinus carpio*

Qusay H. Al-hamadany[1] *Khalidah S. Al-Niaeem*[2]* *Amjed K. Resen*[2]

Received 19/12/2018, Accepted 16/9/2019, Published 1/3/2020

Abstract:

Aqueous extract of poppy plant (*Papaver nudicaule*) with five concentrations (50, 100, 150, 200 and 250) mg/l were used to anesthetize fingerlings of the common carp *Cyprinus carpio* (Mean total length 8.91 ± 0.31 cm and mean total weight 7.72 ± 1.19 gm) instead of the traditional use of MS-222. Results showed that extracted solution of poppy have partial and overall anesthesia effect on these fishes with inverse relationship between the concentrations used and the time needed to reach partial and overall anesthesia, and also direct relationship between concentrations used and time needed for fish recovery. Best results were obtained by using a concentration of 250 mg/l, where time for partial anesthesia was 8 ± 1.52 min., time for overall anesthesia was 10 ± 1.70 min., time needed for partial recovery was 25 ± 2.43 min. and time needed for overall recovery was 35 ± 2.23 min. Fish behavior observations revealed a difference ranging from slow swimming with increasing in breathing rates movements to vertical swimming near the surface, then laying at bottom and too much decrease in breathing rates 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. The results showed also that there were no significant differences ($p \geq 0.01$) in ALP, AST, ALT, LDH and CK among fishes after recovery comparing with the control fishes. Experimental fishes exhibited no stress during anesthesia by using poppy extracted solution. This indicated that the treated fishes exhibited no physiological effects stress which might lead to poor health condition later. The study demonstrated that poppy can be used as an effective anesthetic, as we obtained acceptable induction and recovery times. The poppy can be recommended as suitable anesthetics for fishes (200 or 250 mg/l).

Key words: Anesthetic, Common carp, Hematological effect, Poppy.

Introduction:

Agents, decribed as general anaesthetics, which reversibly depress the sensory centres of the brain to various degrees and which finally eliminate reflex action, are being used more and more widely in fisheries biology (1, 2). General anaesthetics first depress the cortex (stage of analgesia), then the basal ganglia and cerebellum (stage of delirium or excitement), and then the spinal cord (stage of surgical anaesthesia), excessive dosage or prolonged exposure leads to involvement of the medulla; paralysis of the vital respiratory and vasomotor centres is then the usual cause of death, all general anaesthetics act to varying degrees as central nervous system depressants (1, 3).

[1] Marine Science Center, University of Basrah, Basrah, Iraq
[2] Department of Fisheries and Marine Resources, College of Agriculture, University of Basrah, Basrah, Iraq
*Corresponding author: kalidah_salim@yahoo.com
*ORCID ID: 0000-0001-7236-6464

Anaesthetics are often used in aquaculture, fisheries and biological researches as a way to minimize fish hyper-motility, which is a considerable source of injuries during handling procedures (2, 3). The consequent damages from such accidents succumbed fish to increase the susceptibility to pathogens and infection diseases (1, 2, 4). Therefore, reducing fish motility by anaesthetics may decrease the undesirable handling consequences.

Many of the factors that cause stress to fish during aquaculture are the transport, fishing, fish farming intensity and water quality parameters (3) and have significant effects on fish health and their growth (4). To resolve this problem, fish biologists have used a variety of anesthetics in attempts to reduce handling stress (5). Many chemicals have been used in anesthesia in recent years, Tricaine methanesulfonate, Benzocaine, Phenoxyethanol, Isoeugenol, Quinaldine, Propofol, Clove (Carnation

Buds), tobacco, Zataria multiflora, Nicotiana tabacum and *Myrisitca fragrans* (6-11), in order to reduce handling stress in freshwater fish, allowing them to be collected, identified, measured, weighed and subsequently released back to their natural habitats (5, 6). In addition, this anesthetic is used in artificial reproduction and chirurgic processes, as well as with live fish transportation (3) because it mitigates fish stress levels. It also reduces potential negative effects on fish homeostasis, so decreasing mortality rates (4).

An efficacious anesthetic for use with human is Poppy plant (*P. nudicaule*), the active ingredients are Poppy seeds contain small quantities of both morphine and codeine, which are pain-relieving drugs that are still used today (12). There is not enough information on their effects on fish physiology, therefore, in this study, it was designed to determine anesthetic and some hematological effects of poppy on fingerlings common carp, *C. carpio*.

Materials and Methods:

The experiment was conducted on young common carp (*C. carpio*) in which their weights was (7.72 ± 1.19 g), which were brought from the fish farm of Marine Science Center, University of Basrah during the period from November 2017 till January 2018. They were pre-acclimated to laboratory conditions and fed with commercial pellets (Protein 32.56, Lipid 9.11, Carbohydrates 49.52, Moisture 2.88 and Ash 5.93) prior to the anesthesia trials. In this experiment the anesthetics effect of poppy (the plant flower) extract was investigated at five concentrations and control, for each concentration three replicate were used, anesthetics were dosed as followed: 50, 100, 150, 200 and 250 mg/l were prepared according to (13). 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 15.66 ± 0.57° C and pH was 8.2 ± 0.05.

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.

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 plasma was then separated by centrifugation for three minutes at 1500 rpm (14). The levels of serum enzymes were assayed according to the instructions provided with the corresponding enzymatic kits. 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.

The perusal of the result indicates that there is a concentration dependent response in induction and recovery time of poppy used in the present study i.e. the highest concentration of anaesthetic evoked quickest induction and the longest recovery time.

Table (1) shows the induction time of *C. carpio* which decreased with increasing concentrations of poppy, *P. nudicaule*. At 250 mg/ l, the time to reach a complete anaesthesia was 8 min. which is significantly different ($P \leq 0.01$). There was a clear direct relationship between recovery time and concentrations of the anaesthetic, with significant lowered ($P \leq 0.01$). The longest recovery time was 35 min. at a concentration of 250 mg/ l, while the shorter recovery time was 8 min. at a concentration of 50 mg/ l poppy.

Table 1. Effect of poppy on anesthesia and recovery stages (min.) in common carps (Mean ± SD.).

Concentration (mg/ l)	Partial anaesthesia time (min.)	Overall anaesthesia time (min.)	Partial recovery time (min.)	Overall recovery time (min.)
50	52 ± 4.04 [a]	70 ± 5.13 [a]	4 ± 1.15 [a]	8 ± 1.15 [a]
100	28 ± 1.00 [b]	40 ± 5.50 [b]	7 ± 1.50 [b]	12 ± 2.08 [b]
150	16 ± 1.50 [c]	23 ± 2.64 [c]	16 ± 2.52 [c]	23 ± 2.51 [c]
200	12 ± 2.08 [d]	19 ± 1.52 [d]	22 ± 2.00 [d]	29 ± 3.60 [d]
250	8 ± 1.52 [e]	10 ± 1.70 [e]	25 ± 2.43 [e]	35 ± 2.32 [e]
P	0.005	0.002	0.00	0.00

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

The biochemical indices measured in the blood serum of the fish are presented in Table (2). All the anaesthetized groups at all the stages of anesthesia and recovery had no significant (P>0.01) the concentrations of biochemical indices compared with those of the control group. The highest ALP concentration was 59.72 Ul/ l in control and the lowest was 51.5 Ul/ l for fishes anaesthetic by 150 mg/ l of poppy. The highest AST concentration was 5.40 Ul/ l in control and the lowest was 4.60 Ul/ l for fishes anaesthetic by 50 mg/ l of poppy. The highest ALT concentration was 42.75 Ul/l in control and the lowest was 33.25 Ul/ l for fishes anaesthetic by 250 mg/ l of poppy. The highest CK concentration was 77.8 Ul/ l in control and the lowest was 64.2 Ul/ l for fishes anaesthetic by 250 mg/ l of P. nudicaule. The highest LDH concentration was 39.12 Ul/ l in fishes anaesthetic by 250 mg/ l of poppy and the lowest was 30.45 Ul/ l for fishes anaesthetic by 250 mg/ l of poppy.

The poppy at concentration of 200 and 250 mg/l are the most suitable anaesthetizing concentration due to its quick induction of anaesthetization and longer recovery time with the least stress as evident from biochemical indices of common carp.

Table 2: Effect of poppy concentrations on blood serum enzymes of common carp anaesthesia (Mean ± SD.).

Parameter (Ul/ l)	Control	50 mg/ l	100 mg/ l	150 mg/ l	200 mg/ l	250 mg/ l	P
ALP	59.72 ± 3.47[a]	55.23 ± 3.78[a]	54.65 ± 3.25[a]	51.5 ± 2.33[a]	55.3 ± 2.23[a]	54.2 ± 3.23[a]	0.12
AST	42.75 ± 2.54[a]	35.24 ± 3.45[a]	34.4 ± 1.25[a]	34.35 ± 4.02[a]	35.42 ± 4.00[a]	33.25 ± 4.01[a]	0.15
ALT	5.40 ± 0.46[a]	4.60 ± 0.32[a]	4.45 ± 0.32[a]	4.95 ± 0.06[a]	4.22 ± 0.03[a]	4.76 ± 0.04[a]	0.11
CK	33.57 ± 3.24[a]	32.67 ± 3.56[a]	30.45 ±4.41[a]	34.33 ± 3.85[a]	39.12 ± 3.35[a]	32.31 ± 2.32[a]	0.10
LDH	77.8 ± 2.55[a]	73.20 ± 3.76[a]	68.8 ± 4.33[a]	74.5 ± 3.22[a]	71.22 ± 3.02[a]	64.2 ± 2.12[a]	0.10

Similar letters in same row are no significant different (P>0.01).

Discussion:

The prerequisite to use using anaesthetics in the efficient management of aquaculture and during laboratory experimentation is to render the fish immobile so as to make it amenable to manipulation without altering its physiology. However, the choice of anesthetic would not merely depend on the quickest induction and longest recovery time but it is also needs to be ensured that the chosen dose is not so unduly stressful that it may disrupt the normal physiology of the fish and cause histological aberrations in the vital organs.

Fish response to poppy plant exposure varies according to anesthetic concentration. Induction time is inversely related to concentration of poppy plant as the anesthetic is readily absorbed across the gills. The above fact is clearly borne out from the present study where a maximum dose of poppy plant i.e. 200 or 250 mg/l seemingly appears most suitable based on rapid induction of anaesthetization and longest period of recovery, and it is considered most suitable since it does not cause any hematological effects.

The results of the present study has showen that anesthesia time be adversely with concentrations of poppy plant, and there were significant differences (P≤0.01) in anesthesia time between concentrations (50, 100, 150, 200 and 250 mg/l). Also, the results have been revealed relationship between recovery time and anesthetic concentrations, with significant differences (P≤0.01). When using anesthetics, it is predicted that there will be a strong negative correlation between an applied concentration and the time needed to stimulate anaesthesia to the desired stage, as observed previously for several fish species (15-18). Long exposure to anaesthetic will make more absorption of anaesthetic by fish which, in turn, lengthened the recovery time (15).

On the other hand, (19) mentioned that long exposure to anesthetic will make more anesthetic. The statement is not completely ensured otherwise this could be proved with our result due to if it could be said that longer exposure to low concentration of the anesthetic leads to more anesthetic absorption. It can be seggest that short exposure to high anesthetic concentration does it as well. On the other hand, (19, 20) mentioned that compared with anesthesia duration, the recovery time depend on anesthetic concentration. It is believed that the independence of the recovery time from the anesthesia duration, as a result of that anesthetic, has taken by the fish through a diffuse at the gill interface. Therefore, when equilibrium level be done between the gill and anesthetic .the fish no further will take anesthetic, and during recovery, the anesthetic agent is leaked through such gradient. Therefore, the anesthetic concentration controls recovery time but not anesthesia duration (20-22).

The blood is fluid connective tissue of the body in all animals which acts as the main transporting system. In fish it plays vital role like physiological nature of the body with respect to the different parameters of environmental. Parameters of hematological can provide needed information on the physiological status of fishes, and help the aquaculture and research to make proper plans to keep high of the fishes survival. Results of current study have indicated that the 80 min., 50 min., 33, 14 and 9 min. exposure to poppy at different concentrations had no significant differences ($P \geq 0.01$) on the biochemical indices (ALP, AST, ALT, LDH and CK) measured immediately after anaesthesia. Also, there were no significant differences ($P \geq 0.01$) between these biochemical indices for anaesthetic fishes compared with control group (rate of metabolism in the liver is normal). Biochemical indices of blood serum can provide important information about the internal environment of the organism (23-25). Values determined in the present study suggest that internal physiological rolls of organs and tissues of carp are not altered by poppy anaesthesia.

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 not 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.

Based on results of the present study, poppy can be recommended as suitable anesthetics for fishes (200 or 250 mg/l), these concentrations of poppy are the safest and the most effective ones when applied to common carp, instead of the traditional use of MS-222, and does not cause any hematological disruption. MS-222 is an expensive chemical substance that causes cancer for workers and fish consumers and also pollutes the aquatic environments.

It is hoped that the effectiveness of the available anaesthetics for fish will not discourage active testing and development either by "borrowing" from the large and rapidly expanding array of anaesthetics for humans or by modifying the structure of functional groups of the present anaesthetics. Research in anaesthesiology is leading to more precise control and selection of the type and level of anaesthesia, advances which should be exploited in fisheries biology.

Conflicts of Interest: None.

The author has signed on animal welfare statement.

References:

1. Bell GB. A guide to the properties, characteristics, and uses of some general anaesthetics for fish. Fisheries Research Board of Canada Sir Charles Tupper Building Ottawa 8, Ontario, Canada, 1967; p. 19.
2. Sehdev HS, Mcbride JR, Fagerlund UHM. 2-Phenoxyethanol as a general anaesthetic for sockeye salmon. J. Fish. Res. Bd. Canada 1963; 20: 1435-1440.
3. Weinert NC, Julieta V, Ádson C, Rozyanne RA, Aldo CO, Cláudio RSM et al. Hematology of Nile tilapia (*Oreochromis niloticus*) subjected to anesthesia and anticoagulation protocols. Semina: Ciências Agrárias, 2015; 36(6 Suppl. 2): 4237-4250.
4. Lepic P, Stara A, Turek J, Kozak P, Velisek J. The effects of four anaesthetics on haematological and blood biochemical profiles in vimba bream, *Vimba vimba*. Vet. Med. 2014; 59: 281-287.
5. Barata M, Sores F, Aragao C, Almeida AC, Pousao-Ferreira P. Efficiency of 2-phenoxyethanol and clove oil for reducing healing stress in reared meager, *Argyrosomus regius* (Pisces: Sciaenidae). J. World Aquacult. Soc. 2016; 47: 82-92.
6. Adewale AY, Adeshina I, Yusuf OY. Anaesthetic effect of ocimum gratissimum extract on *Oreochromis niloticus* juveniles. European J. Exp. Biol. 2017; 7(2): 1-4.
7. Zargham D, Sharif Rohani M, Falahati Naserabad I, Bashti T. Investigation of anesthetizing effect of tobacco (*Zataria multiflora*) aqueous and alcoholic extract on rainbow trout (*Oncorhynchus mykiss*). Iranian Sci. Fish. J. 2013; 21: 33-40.
8. Aliakbar Y, Hadideh Narges **J.** Determination of MS222 Anesthetic Dose and Its Effect on Liver and Kidney Tissues of *Barbus grypus*. 2nd Int'l Conference on Advances in Environment, Agriculture and Medical Sciences (ICAEAM'15). June 11-12, Antalya (Turkey) 2015.
9. Santos S, Ghanawi J, Saoud I P. Effects of water temperature and body weight on anaesthetic

efficiency in marbled rabbitfish (*Siganus rivulatus*). Aquacult. Res. 2015; 46: 928-936.
10. Baiju AP, Benno Pereira FG, and Jayaprakas V. Assessment of clove oil and Benzocaine anaesthesia on haematological and histopathological profile of *Haludaria fasciata*. J. Bio. Env. Sci. 2017; 10(4): 27-37.
11. Al-Niaeem KS, Mohammed FA, Al-hamadany QH. The anaesthetic effect of Nutmeg powder, *Myrisitca fragrans* on young common carp, *Cyprinus carpio*. Biol. Appl. Environ. Res. 2017; 1(2): 279-286.
12. Meadway C, George S, Braithwaite R. Opiate concentration following the ingestion of poppy seed products – evidence for 'the poppy seed defence'. Forensic Sci. Int. 1998; 96: 29-38.
13. Al-Niaeem KSK. Infection distribution of fish parasites in Basrah province and pathological effects of *Saprolegnia* sp. and its susceptibility to some plant extracts. Ph. D. Thesis, Coll. Agric., Univ. Basrah 2006; 172 pp. (In Arabic).
14. Yang JL, Chen HC. Serum metabolic enzyme activities and hepatocyte ultrastructure of common carp after gallium exposure. Zool. Stud. 2003; 42(3): 455-461.
15. Fernandes IM, Bastos YF, Barreto DS, Lourenço LS, Penha JM. The efficacy of clove oil as an anaesthetic and in euthanasia procedure for small-sized tropical fishes. Braz. J. Biol. 2017; 77(3): 444-450.
16. Zahl IH, Samuelsen O, Kiessling A. Anesthesia of farmed fish: implications for welfare, Fish Physiol. Biochem. 2012; 38: 201-218.
17. Hoseini SM, Mirghaed AT, Yousefi M. Application of herbal anaesthetics in aquaculture. Reviews in Aquaculture 2019 Aug.; 11(3):550–564.
18. Feng G, Zhuang P, Zhang L, Kynard B, Shi X, Duan M, et al. Effect of anaesthetics MS-222 and clove oil on blood biochemical parameters of juvenile *Siberian sturgeon* (*Acipenser baerii*). Journal of Appl. Ichthyol. 2011; 27: 595-599.
19. Mortazevi Zadeh S A, Peyghan, R, Yooneszadeh Feshalami M, Sharifiyan M. Determine of appropriate concentration of propofol anesthetic drug in Benni *(Barbus sharpeyi)*. Iranian Sci. Fish. J. 2012; 21(2): 133-142.
20. Pawar HB, Sanaye SV, Sreepada RA, Harish V, Suryavanshi U, Ansari TZA. Comparison of efficacy of four anesthetic agents in the yellow seahorse *Hippocampus kuda* (Bleeker, 1852). Aquaculture 2001; 311(1-4): 155-161.
21. Kamble AD, Saini VP, Ojha, ML. The efficacy of clove oil as anesthetic in common carp (*Cyprinus carpio*) and its potential metabolism reducing capacity. Int. J. Fauna Biol. Stud. 2014; 1(6): 1-6.
22. Weyl O, Kaiser H, Hecht T. On the efficacy and mode of action of 2-phenoxyethanol as an anaesthetic for goldfish, *Carassius auratus* (L.), at different temperatures and concentrations. Aquacult. Res. 1996; 27(10): 757-764.
23. Bahrekazemi M, Yousefi N. Plasma enzymatic, biochemical and hormonal responses to clove oil, 2-phenoxy ethanol, and MS-222 exposed to Caspian brown trout (*Salmo trutta caspius*, kessleri). Iranian J. Aquat. Anim. Health 2017; 3(1) 47-60.
24. Kristan J, Stara A, Turek J, Policar T, Velisek J. Comparison of the effects of four anaesthetics on haematological and blood biochemical profiles in pikeperch (*Sander lucioperca* L.). Neuroendocrinol. Letter 2012; 33(Suppl. 3): 66-71.
25. Al-Niaeem KS, Al-hamadany QH, Mohammed FA. Using aqueous extract of *Myristica fragrans* Houtt for anaesthesia of common carp (*Cyprinus carpio* L.) fry. Euphrates J. Agricult. Sci. 2017; 9(4): 1454 – 1448.

فعالية مستخلص نبات الخشخاش *Papaver nudicaule* لتخدير أسماك الكارب الشائع *Cyprinus carpio*

قصي حامد الحمداني[1] خالدة سالم النعيم[2] أمجد كاظم رسن[2]

[1] مركز علوم البحار، جامعة البصرة
[2] قسم الأسماك والثروة البحرية، كلية الزراعة، جامعة البصرة

الخلاصة:

استعملت خمسة تراكيز (50 و100 و150 و200 و250) ملغم/ لتر من المستخلص المائي لنبات الخشخاش *Papaver nudicaule* 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 للخشخاش 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 250 ملغم/ لتر، إذ كان معدل وقت التخدير الجزئي 8 ± 1.52 دقيقة، وبلغ معدل وقت التخدير الكلي 10 ± 1.70 دقيقة، أما معدل وقت الإفاقة الجزئي فكان 25 ± 2.43 دقيقة، وبلغ معدل وقت الإفاقة الكلي 35 ± 2.23 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 إضطجاع السمكة على القاع بالإضافة إلى بطئ في التنفس. بينت النتائج عدم وجود اختلافات معنوية ($p \geq 0.01$) في فعالية أنزيمي ناقل الأسبارتيز (AST) وناقل الألنين (ALT) والفوسفاتيز القاعدي (ALP) واللاكتيك ديهايدروجينيز (LDH) والكرياتين كاينيز (CK) في الأسماك بعد الإفاقة الكلية مقارنة بأسماك السيطرة، وهذا يدل وهذا يدل على عدم تعرض الأسماك إلى الإجهاد نتيجة تخديرها بالمستخلص المائي لنبات الخشخاش ولا توجد تأثيرات فسلجية تؤدي إلى ضعف حالتها الصحية، مما قد يؤدي إلى تدهور الحالة الصحية في وقت لاحق .أظهرت الدراسة أنه يمكن استخدام الخشخاش كمخدر فعال، حيث حصلنا على أوقات تخدير واسترداد مقبولة.، يمكن التوصية بنبات الخشخاش كمادة مخدرة مناسبة للأسماك (200 أو 250 ملغم/ لتر).

الكلمات المفتاحية: تخدير، أسماك الكارب الشائع، تأثيرات دمية، نبات الخشخاش.