Available online at www.jpsscientificpublications.com
Volume – 8; Issue - 4; Year – 2022; Page: 2627 – 2633
DOI: 10.22192/iajmr.2022.8.4.5

Indo – Asian Journal of Multidisciplinary Research (IAJMR)
ISSN: 2454-1370

RUTA (*Ruta montana*) TO ANESTHETIZE ON COMMON CARP *Cyprinus carpio* JUVENILES

Khalidah S. Al-Niaeem[1*], Amjed K. Resen[1] and Majid M. Taher[2]

[1]*Department of Fisheries and Marine Resources, College of Agriculture, University of Basrah, Iraq*
[2]*Aquaculture Unit, College of Agriculture, University of Basrah, Basrah, Iraq*

Abstract

Extracted solution of Ruta (*Ruta montana*) with five concentrations (200, 400, 550, 650 and 750 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 Ruta has 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 750 mg/l, where time for partial anesthesia was 6 ± 1.25 min., time for overall anesthesia was 9 ± 1.70 min., time needed for partialrecovery was 28 ± 2.51 min. and time needed for overall recovery was 37 ± 2.64 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 Ruta extracted solution. The results showed that there were no significant differences ($p>0.01$) in both AST and ALT among fishes after recovery in comparison with the control fishes. This indicated that the treated fishes exhibited no physical damage which might lead to poor health condition.

Key words: Ruta (*Ruta montana*), Common carp *Cyprinus carpio*, Anaesthetize and Juveniles.

1. Introduction

Anesthesia is very important in fish farms to reduce stress and damage during fish handling (weighing, grading, transport and blood sampling) (Küçük and Çoban, 2016). Anesthesia inhibits autonomic activity and reduces muscle contraction, so high doses or prolonged exposure to the anesthetic will lead to a breakdown in respiration and circulation (Dziaman *et al*., 2005). Several anesthetics have been used to anesthetize fish such as MS222 (Tricaine methanesulfonate), Benzocaine, Etomidiate, Metomidiate, Phenoxiethanole, Equinaldine and Equinaline Sulphate (Mercy *et al*., 2013). There are many considerations that must be taken before using a anesthetics for fish, including the efficiency of the anesthetics and the possibility of obtaining it. As well as being safe and inexpensive (Mylonas *et al*., 2005). The response of fish to anesthesia and the amount of dose depends on many important things, including age, sex and maturity, as well as biological factors such as temperature, salinity and others (Sneddon, 2012). In recent years, anesthetics have been turned to extracted from medicinal herbs, such as the aqueous extract of (*Peganum harmala*) (Abdel-Fattah *et al*., 1997)

***Corresponding author*:** Khalidah S. Al-Niaeem
***Received*:** 26.04.2022; ***Accepted*:** 15.05.2022;
***Published*:** 13.06.2022.

and clove oil, which is extracted from the stems, leaves and buds of the clove plant (Wagner *et al*., 2003; Inversen *et al*., 2003). Locally some plant extracts have been used as anesthetics for fish, including the study of Al-Niaeem *et al*. (2017a) and Al-Niaeem *et al*. (2017b) used aqueous extract of nutmeg in the anesthesia of juveniles of common carp, Al-Niaeem *et al*. (2019) using of anise (*Pimpinella anisum*) and chamomile (*Matricaria chamomilla*) powders in the anesthesia of common carp and Resen *et al*. (2020) using poppy, *Papaver nudicaule* extract as an anesthetic for the common carp.

The current experiment aimed to: i) Replacing the carcinogenic substance MS-222 with substances that are safe for human health; ii) Find cheap, year-round alternatives to anesthetizing fish such as ruta; iii) Reducing economic losses in fish exposed to anesthesia due to deaths or malformations that may occur later; iv) Reducing the costs of sanitary measures due to the lack of limitations during the use of aqueous extract of Ruta to anesthetize fish; v) Determining the effect of the aqueous extract of solute on stress of anesthetized fish by measuring the concentration of glucose in the blood plasma, as well as demonstrating the extent of its effect on fish health by measuring the activity of the enzyme aspartate transporter (AST) and alanine transporter (ALT) in the serum of fish.

2. Materials and Methods

Common carp were brought from the ponds of the Marine Science Center - University of Basra (Average total length 8.91 ± 0.31 cm and average total weight 7.72 ± 1.19 g). The fish were placed in the laboratory for 72 hours to acclimatize in a glass container (30 × 30 × 50) cm (30 fish and 10 other fish as stock in case of fatalities in the anesthesia experiments). The water temperature was (15.66 ± 0.57) 0 °C and the pH was (8.83 ± 0.05). A stopwatch has been used to the nearest minute. An appropriate amount of Ruta (leaves and flowers) was taken.

It was finely ground using an electric grinder. Then the required concentrations were prepared in the experiment (200, 400, 550, 650 and 750 mg/L) after dissolving in hot water. It was left for an hour to extract the total active substances, and then filtered to get rid of the suspended substances (Figure - 1) (Twaij *et al*., 1983) and modified by Al-Niaeem (2006). Then other glass container for anesthesia experiments, the dimensions of which are (24 × 14 × 10) cm. Six fishes in each concentration (three replicates for each concentration) were used to perform the anesthesia experiments, while observing the behavior of the fish to reach the partial stress condition in addition to reaching the total stress condition. As well as monitoring the fish up to a state of partial and total recovery. The concentration of glucose in the blood plasma was measured using a laboratory kit prepared by the French company Biomaghreb with a spectrophotometer at a wavelength of 505 nm according to the following equation:

Glucose concentration (mg/100 ml) = (sample reading/standard reading) × 10

The activity of the aspartate amino transferase (AST) enzyme was estimated at a wavelength of 546 nm in a spectrophotometer by means of a ready-made estimation kit produced by Randox Company. As for calculating the concentration, it was the result of the difference between the two readings for one model (reading the test – reading the control). The difference between readings gives a certain value that is converted into the corresponding using a table installed in the instructions for the work kit, where the concentration is in the standard unit (international unit/liter). The activity of the alanine transporter enzyme was estimated by means of a ready-made estimation kit produced by Randox Company by means of a spectrophotometer at a wavelength of 546 nm. As for calculating the concentration, it was the result of the difference between the two readings of the same model (the test reading - the control reading), as the difference between readings gives a certain value that is converted into the corresponding using a table installed in the instructions for the work kit, where the concentration is in the standard unit (international unit/liter).The statistical program was used in the statistical analysis SPSS

Statistics V. 19 to test the differences between the means for all 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.

Figure - 1: ***Ruta montan***

3. Results

All concentrations used for partial and total anesthesia on fish showed a variation in the time of partial and total anesthesia, partial and total recovery. The concentration of 750 mg/L recorded the best time for general anesthesia and total recovery. The average partial anesthesia time ranged from 6 ± 1.25 minutes, The average total anesthesia time was 9 ± 1.70 minutes, while the concentration of 200 mg/L was characterized by the length of access to partial and total anesthesia 62 ± 4.04 and 80 ± 5.13 minutes, respectively. However, the total recovery period was the shortest among the concentrations used for the aqueous extract, 8 ± 1.15 minutes. Significant differences ($p<0.01$) were found in the partial and total anesthesia and the partial and total recovery of common carp among the five anesthetics concentrations (Table - 1).

The Figure - 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 occurs with rapid movements and in different directions and escape when trying to catch it and the number of movements of the operculum increases significantly and then begins to decrease. Then, a decrease in the movement of the caudal fin, and total anesthesia occurred by the stability of the fish on the bottom of the tank and simple positional movements and ease of catching. The fish are placed at the bottom of the container in a lateral position, a very large decrease in the movement of the Operculum, 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

There were no significant differences ($p>0.01$) for the concentration of glucose in the blood plasma of fish after the total recovery condition after comparing with the control fish (Table - 2).

The activity of the enzyme aspartate and alanine transporter

There were no significant differences ($p>0.01$) for the concentration of AST and alanine transporter in the serum of fish after total recovery after comparing with control fish (Table 3).

Table - 1: Effect of Ruta on anesthesia and recovery stages (min.) in common carps

Concentration (mg/l)	Partial anaesthesia time (min.)	Overall anaesthesia time (min.)	Partial recovery time (min.)	Overall recovery time (min.)
	(Mean ± SD.)*			
200	62±4.04 [a]	80±5.13 [a]	4±1.14 [a]	8±1.15[a]
400	38±1.00[b]	50±5.50 [b]	7±5.51 [b]	12±2.08[b]
550	18±1.50	33±2.64 [c]	16±2.64 [c]	23±2.51[c]
650	[d]10±2.08	14±1.52 [d]	22±2.00 [d]	29±3.60[d]
750	[e]6±1.52	9±1.71 [e]	28±2.51 [e]	37±2.64 [e]

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

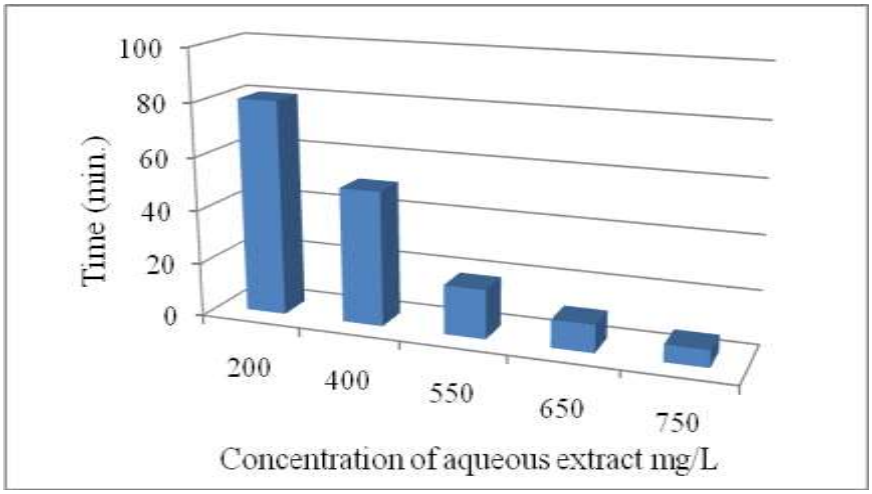

Figure - 1: Effect of using different concentrations of aqueous extract on overall anesthesia Ruta for common carp juveniles

Table - 2: The concentration of glucose in the blood plasma of common carp fishes subjected to anesthesia with aqueous extract of Ruta of overall recovery

Concentration (mg/L) (Mean ± SD.)						*Parameter (mg/100ml)*
750	**650**	**550**	**400**	**200**	***control***	
60.01 [a]3.24±	± 64.63 [a] 2.01	61.80 [a] 2.20±	*60.53 ±2.17[a]*	*57.41 ±3.10[a]*	54.67 *±4.08[a]*	*Glucose*

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

Table - 3: The concentration of aspartateand alanine in the blood plasma of common carp fishes subjected to anesthesia with aqueous extract of Ruta of overall recovery.

Concentration (mg/L) (Mean ± SD.)						*Parameter (mg/100ml)*
750	**650**	**550**	**400**	**200**	***control***	
56.21 [a]3.24±	±54.03 [a] 4.21	56.10 [a] 2.25±	*50.03 ±3.77[a]*	*44.31 ±2.60[a]*	40.24 [a]2.43±	*AST*
6.11 [a]0.24 ±	6.32 [a]0.21 ±	6.10 [a]0.22±	*5.53* [a]2.07±	*6.01± 0.30[a]*	± 5.67 *0.08[a]*	*ALT*

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

4. Discussion

Anesthetics were used to reduce the negative effects of physiological stress on fish in various aquaculture activities (Gholipour *et al*., 2011). The rue herb is used effectively for some autonomic nervous system disorders. Its oil is used externally to treat skin diseases, especially skin infections and eczema, to relieve muscle pain, nerve pain, spasms, and rheumatic pain, and to relieve tonsillitis and gingivitis (Paulini, 1987).The use of appropriate concentrations of aqueous extract of rue showed positive results in anesthetizing fish, and Ruta plant is considered one of the safe, cheap and effective materials. The anesthetic reason in Ruta is due to alkaloids, which are organic substances that have distinctive physiological properties, some of which are tonic, some of which are anesthetic, and some of which are sedative and analgesic. The Ruta plant contains Codeine ($C_{18}H_21NO_3$). This acts as a simple analgesic (Adamska-Szewczyk *et al*., 2016; Rutaaceae, 2017). The results of the follow-up of the behavior of carp fish also showed In the anesthetic solution of the Ruta plant, the fish went through three phases of anesthesia: a decrease in the activity of the fish and an increase in the respiratory rate, the appearance of signs of loss of balance with the start of vertical swimming and the decrease in respiratory movements, and finally turn to the side and decrease in respiratory movements.

The results of the current study showed a direct relationship between the concentration of the anesthetic substance used and the time of occurrence of partial and total anesthesia, While he recorded an inverse relationship with the time of partial and total recovery, and this is consistent with what was made by Sado (1985) in his study on some types of tilapia fish and the effect of the Quinaldine, It also agrees with what was indicated by Pirhonen and Hoskonen (2006) in their study of the effect of clove oil doses in anesthetizing tilapia fish and Al-Niaeem *et al*. (2017) in their study of the effect of nutmeg on the anesthesia of common carp fish juveniles, And the existence of an inverse relationship between the time of total anesthesia and the time of total recovery. The physiological characteristics of a living organism, including fish, express the internal state of the body. It is one of the scientific criteria that reflect positively or negatively on health indicators in fish. Therefore, glucose in the blood plasma is an important factor.

The concentration of glucose in the current study did not differ in juveniles carp fish after total recovery when compared to the control treatment. This positive result indicates that the fish did not suffer from stress by use anesthetic (Martinz-Porchas *et al*., 2009). Also, the concentration of fish liver enzymes (aspartate transporter and alanine transporter) did not differ in the current study after the total recovery of fish when compared to the control treatment. t confirms that the use of the aqueous extract of Ruta did not affect the health of juveniles common carp fish, and the use of rue did not affect the increase or decrease of liver enzymes, especially the enzymes of aminotransferases. The aqueous extract of Ruta can be used in fish hatcheries during the process of artificial propagation to reduce injuries, bruises and wounds that affect fish during the processes of handling and injecting them with the hormone or during the process of collecting eggs and sperm, thus reducing the economic losses resulting from this.

5. References

1) Abdel-Fattah, A. F. M.; Matsumoto, K. and Murakami, Y. (1997). Central Serotonin level - dependent changes in body temperature following administration of tryptophan to party-liner- pretreated rats. Gen. Pharmacol., 28: 405 -409.
2) Al-Niaeem, K. S. (2006). Infection distribution of fish parasites in Basrah province and pathological effects of *Saprolgnia* sp. and its susceptibility to some plant extracts. Ph. D. Thesis Coll. Agric., Univ. Basrah: 172pp. (In Arabic).
3) Al-Niaeem, K. S.; Alhamadany, Q. H. and Mohammed, F. A. (2017a). Using aqueous extract of *Myristica fragrans* Houtt for anaesthesia of common carp

(*Cyprinus carpio* L.) fry. Euphrates J. Agricult. Sci., 9(4): 1454 - 1448.

4) Al-Niaeem, K. S.; Mohammed, F. A and Alhamadany, Q. H. (2017b). The Anaesthetic Effect of Nutmeg Powder, *Myrisitca fragrans* on Young Common Carp, *Cyprinus carpio*.Biol. App. Environ. Res., 1(2): 279-286.
5) Al-Niaeem, K.S.; Abdulrahman, N.M.A. and Attee, R.S. (2019). Using of anise (*Pimpinella anisum*) and chamomile (*Matricaria chamomilla*) powders for common carp *Cyprinus carpio* L. anesthesia. Biol. Appl. Environ. Res., 3(2): 111-117.
6) Resen, A.R.; Al-Niaeem, K.S. and Al-Hasson, H.A. H. (2020). Assessment of lemon balm (*Melissa officinalis*) on common carp, *Cyprinus carpio*: Anesthesia. Life Science Archives, 6(1): 1749-1754.
7) Dziaman, R.; Kyszejko, B. and Hajek, G. (2005). The effects of MS-222 on the cardiac and respiratory function and behaviour of common carp, *Cyprinus carpio* L., during general anaesthesia. Acta Ichthyologicaet Piscatoria, 35: 125–131.
8) Gholipour, K. H.; Mirzargar, S. S.; Soltani, M.; Ahmadi, M; Abrishamifar, A; Bahonar, A. and Yousefi, P. (2011). Anesthetic effect of tricaine methane sulfonate, clove oil and electro anesthesia on lysozyme activity of *Oncorhynchus mykiss*. Iranian J. of Fish. Sc., (10): 393-402.
9) Hoskonen, P.; Pirhonen, J. (2006). Effects of repeated handling, with or without anesthetics, on feed intake and growth in juvenile rainbow trout, *Oncorhynchus mykiss* (walbaum). Aquaculture, 37(4): 409 - 415.
10) Inversen, M.; Finstad, B.; McKinley, R. S. and Eliassen, R. A. (2003). The efficacy of etomidate, clove oil, Aqui-STM and Benzoak® as anaesthetics in Atlantic salmon (*Salmo salar* L.) smolts, and their potential stress-reducing capacity. Aquaculture, 221: 549-566.
11) Küçük1, S. and Çoban, D. (2016). Effects of Tricaineas an anaesthetics on goldfish, *Carassius auratus* (Linnaeus 1758) at different salinities and concentrations. Turkish J. Fish. Aqu. Sci., 16: 605-610.
12) Martinz-Porchas, P; Martinez-Cordova, L. R. and Ramos-Enriquez, R. (2009). Cortisol and glucose: Reliable indicators of fish stress. Pan-Am. J. Aqua. Sci., 4(2): 158-178.
13) Mercy, T.V.A.; Malika, V. and Sajan, S. (2013). Use of tricaine methane sulfonate (MS-222) to induce anaesthesia in *Puntius denisonii* (Day, 1865) (Teleostei: Cypriniformes: Cyprinidae), a threatened barb of the Western Ghats, India. J. Threatened Taxa, 5:(9): 4414-4419.
14) Mylonas, C.C.; Cardinaletti, G.S.I. and Polzonetti, M.A. (2005) Comparative efficacy of clove oil and 2-phenoxyethanol as anaesthetics in the aquaculture of European sea bass (*Dicentra rchuslabrax*) and gilthead seabream (*Sparus aurata*) at different temperatures. Aquaculture, 24(6): 467-481.
15) Paulini, H.; Eilert, U. and Schimmer, O. (1987). Mutagenic compounds in an extract forma rutaeherba (*Ruta graveolens* L) Mutagenicity is partially causesd by furoquinoline alkaloids. Mutagenesis, 2(4):271-3
16) Rutaceae, A. (2017). Rutagraveolens, a useful homeopathic medicine for musculoskeletal disorders. La Revue d'Homéopathie. 8(2): 23-26.
17) Sado, E.K. (1985). Influence of the anaesthetic quinaldine on some tilapias. Aquaculture, (46): 55-62.
18) Sneddon, L. (2012). Clinical anesthesia and analgesia in fish. J. Exotic Pet Med., 21(1):32-43.
19) Twaij, H.A.A.; Kery, A. and Al-Khazragi, N. K. (1983). Some pharmacological, toxicological and photochemical

investigation on *Centaure phyllocephala*. J. Ethopharmacol., 9: 47-52.

20) Wagner, G.N.; Singer, T.D. and McKinley, S.R. (2003). The ability of clove oil and MS-222 to minimize handling stress in rainbow trout (*Oncorhynchus mykiss* Walbaum). Aqua. Res., 34: 1139-1146.

Access this Article in Online

Quick Response Code	
Website	**www.jpsscientificpublications.com**
DOI Number	**DOI: 10.22192/iajmr.2022.8.3.4**
Thomson Reuters Researcher ID	**K-4194-2016**
ISI Impact Factor	**3.652**

How to Cite this Article:

Khalidah S. Al-Niaeem, Amjed K. Resen and Majid M. Taher. (2022). Ruta (*Ruta montana*) to Anesthetize on Common Carp *Cyprinus carpio* JUVENILES. *Indo - Asian Journal of Multidisciplinary Research*, 8(4): 2627 – 2633.

DOI: 10.22192/iajmr.2022.8.3.5