

# The Effectiveness of Ketamine as an Anesthetic for Fish (Rainbow Trout – *Oncorhynchus mykiss*)



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## Abstract

Ketamine was evaluated as water-soluble anesthetics drug for a species of fish, rainbow trout (*Oncorhynchus mykiss*). Fish (size ~20 - ~240 gr.) were exposed to a 100-ppm concentration of Ketamine solution (dissolved in water), they were arranged in 4 treatments based on their weight range (Treatment-1= 22.8±3.4 g; Treatment-2= 51.7±4.4 g; Treatment-3= 69.8±5.2 g and Treatment-4= 243.8±20.7 g). Elapsed time for anesthesia duration (stage1 to 3) and recovery duration was recorded. Also, surveillance was evaluated after recovery. Ketamine was effective to cause anesthesia in the fish as 100 ppm concentration. 10 fishes of each treatment (%100) were anesthetized and were induced in stageIII-Plane3 of anesthesia within 2-3 min after exposure to anesthetic solution (Treatment-1= 110.3±3.5 seconds; Treatment-2= 140.0±5.9 sec; Treatment-3= 180.0±5.8 sec and Treatment-4= 190.0±5.8 sec). Anesthesia lasted for 5-6 min while fish were immersed in clean water (water without drug). So, Anesthesia continued for about 5-6 minutes (Treatment-1= 370.3±10 seconds; Treatment-2= 329.6±22 sec; Treatment-3= 300.0±12 sec and Treatment-4= 280.0±5 sec). All fish were alive after recovery. The result shows that Ketamine can produce anesthesia in the fish as a water-soluble drug and it is safe and effective. It may show that Ketamine can be absorbed through gills and can reach the nervous system of fish by blood circulation. The anesthesia duration in every treatment was showed a significant difference compared with other treatments ( $p<0.05$ ).

**Keywords:** Fish; Ketamine; Anesthesia; Aquaculture; Pharmacology

## Introduction

Anesthetics are necessary for reducing stress and strife in fish for a variety of activities ranging from transporting to surgical operations also it is important for sampling [1-3], egg extraction (artificial reproduction), drug or vaccine injection, physical examination, blood sampling, biopsy, or biometry for aquatic animals. The efficiency, safety and harm of anesthetics compound depend on fish species, fish size, physicochemical properties of water (water quality), environmental factors (temperature), type of anesthetic drug and drug delivery method [4-6].

There are different compounds as anesthetics in fish, such as Ether [7], Tricaine methanesulfonate (MS-222, Fiquel) [8-10], Quinaldine (2-methyl-quinoline) [9-12], Sodium Bicarbonate [13,14], Metomidate, Benzocaine, Chrobutanol, Clove oil, Eugenol, Iso-eugenol, 2-Phenoxyethanol, Quinaldine-sulphate [10,15-18], 4-styrylpyridine (4-SP) [19], Carbon dioxide, Salt (NaCl), Etomidate, Chlorotone, Chlorobutanol, Tobacco juice (Nicotine) [1], Clove, Diazepam, Chloral hydrate, Alphaxalone, Halothane,

Ethanol, Lidocaine, Methyl-p-Phenol, pentobarbitone, Procaine, Propanidid, Na-amital, etorphine [20]. Some of them are effective while others have ambiguous anesthetic effects.

Clove and its essence are applied as anesthetic in some aquatic animals [3,17]. Clove is an herb which causes anesthesia in the fish as natural form (flower bud) [3,17,20]. In IRAN (in hatcheries), dry flower buds of the clove tree are ground and used as anesthetic in fish (3-30 grams of clove powder in 20 liters of water). It can lead to anesthesia in small and large fish up to 5 Kg effectively. Clove is an organic, natural, not expensive anesthetic in rainbow trout. Anesthetics could be divided into two categories of synthetic (chemical) and natural (plant-based) products [21]. Some of anesthetics are effective while others have ambiguous anesthetics effects.

KETAMINE ("Ketalar™": 2-[o-chlorophenyl]-2-[methylamino] cyclohexanone hydrochloride) is a rapid-acting non-barbiturate general anesthetic from 1960s. Its structure is in Figure 1. The

drug can be given intravenously or intramuscularly for major or minor surgery in medicine and veterinary [22-25]. The first clinical study on humans was conducted in 1964 [26]. In human, Ketamine is a P450 substrate drug (2B6) that blocks the NMDA subtype of glutamate ionotropic receptors and increases systemic blood pressure without significant respiratory depression. Its induction dose is "1-2 mg/Kg IV" with 5-10 min duration of action and its clearance is 12-17 mL/Kg/min in man. Ketamine

bounds with serum protein about %12. Its half-time elimination is 2-4 hours. Ketamine is a partially water-soluble and highly lipid-soluble phencyclidine derivative. It differs from most other intravenous anesthetics in that it produces significant analgesia [27]. The objective of this study is to determine anesthesia effect of ketamine in soluble form in water on rainbow trout fish (Figure 1).

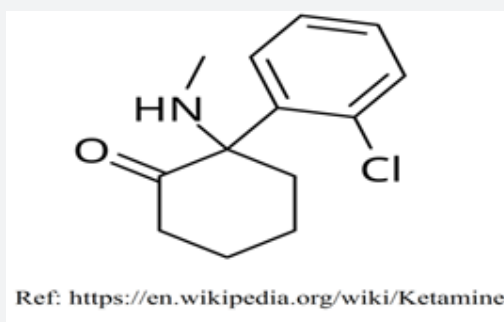


Figure 1: Chemical structure of Ketamine.

## Material and Methods

### Fish

Fish species was Rainbow trout (*Oncorhynchus mykiss*). They were small size ranging from 18-257 grams. Fish were captured from several ponds. They were sorted in 4 weight groups (treatments) (Table 1). Each treatment was conducted in a plastic basin in water.

### Drug

Ketamine was applied as anesthetic drug. Exactly, 1.3 gram of pure powder of Ketamine (Hospital grade) was dissolved in 13 liters of fresh spring water. Its final concentration was 100 ppm.

### Water quality

Properties of spring water (fresh-water) was 11°C, pH=7.6, with dissolved oxygen of 6.5 mgL<sup>-1</sup>. The trials were started at 11:00 AM-0'clock.

### Method

For each treatment, 10 fish were caught and were put up in a basin containing anesthetic solution (100 ppm), and the researcher started to record the time. Each stage (1-4) of anesthesia in the fish was recorded. Then (after stage III plane3; fish settlement stage), 10 anesthetized fish were transferred to a basin with fresh water without drug and anesthesia duration and full recovery duration were recorded (Table 1). This stage was done for each treatment of fish. After 1 hour, the number of live fish was counted in each treatment.

## Procedures

To evaluate the effect of anesthetic drug on rainbow trout, four treatments (groups) of fish were prepared. Each treatment contained 10 fish. These four treatments had different weight ranges. After catching, the fish were kept in a tank of fresh water for adaptation to the new condition for testing. Then 4 pans (basin) were taken. An aqueous solution of the drug was prepared separately in 4 pans. The final drug concentration was 100 ppm in each pan. In each pan, 12 liters of spring water was poured. Temperature, salinity, dissolved oxygen, and pH of water were measured in each pan. Then 13 g of anesthetic drug was dissolved in one liter of water and it was added to a pan. This was done for each pan repeatedly. The 10 fish of the first treatment were transferred to the first pan. The timing was done immediately. After transfer, the drug affected the fish, and gradually the apparent signs of anesthesia were observed in the fish, such as the rapid movement of the gill's operculum, unbalanced swimming, decreased mobility, and final settlement.

At first, the fish lost their balance and started to swim irregularly, and then they were swimming almost on their body side, but they were still able to swim and showed little activity, and finally, after a while, they became completely inactive and anesthetized. They settled at bottom of the pan without movement. Currently, it was easy to touch them without fish reflex. The time required for each important step of anesthesia was recorded. The fish were then transferred to another pan containing spring water, which lacked anesthetic drug. Failure to meet the anesthetic caused the fish to gradually return to consciousness and they

recovered finally. Approximately, three stages of recovery were identified in the fish (Table 2). On the other hand, we found 3 stages for recovery from anesthesia to normal condition in the

fish. The duration of each stage was recorded, Figure 2. This operation was repeated independently for each treatment of fish.

**Table 1:** Results recorded by the effect of “Ketamine” as anesthetic in rainbow trout.

Number of Column															
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Treatment	Fish number	Fish Genus Sp.	Mean of fish weight (Grams)	Minimum of fish weight (Grams)	Maximum of fish weight (Grams)	Ketamine concentration (ppm) in water	Start time <sup>2</sup>	Elapsed time to cause Stage <sup>3</sup> II of anesthesia (Seconds)	Elapsed time to cause Stage III-Plane I of anesthesia (Seconds)	Elapsed time to cause Stage III-Plane2 anesthesia (Seconds)	Elapsed time to cause Stage <sup>4</sup> III-Plane3 anesthesia (Seconds) [Muscle relaxation]	Efficiency (Percent of fish which had been anesthetized)	Anesthesia duration <sup>5</sup> (Seconds)	Full recovery duration <sup>6</sup> (Seconds)	Total duration <sup>7</sup> (Seconds)
1	10	OM <sup>1</sup>	22.8 ± 3.4	18	29	100	0	24.1± 0.3	60.3± 0.7	80± 3.3	110.3± 3.5	100	370.3± 10	537.3± 41.2	647.6
2	10	OM	51.7 ± 4.4	46	58	100	0	30.2± 0.9	59.7± 2.9	89.8± 4.1	140± 5.9	100	329.6± 22	512± 26.7	652
3	10	OM	69.8 ± 5.2	63	76	100	0	32.4± 1.3	89.8± 4.3	120± 5.5	180± 5.8	100	300± 12	620± 18.5	800
4	10	OM	243.8 ± 20.7	206	259	100	0	37.4± 1.9	90± 4.9	135± 7.5	190± 5.8	100	280± 5	757± 64.7	947

<sup>1</sup>*Oncorhynchus mykiss* (Rainbow trout); <sup>2</sup>Start time, is the time that fish were entered into the basin containing water and dissolved anesthetic; <sup>3</sup>Anesthesia Stages (Sneddon, 2012); <sup>4</sup>Fish have been relaxed at the bottom of the basin and have no movement. They did not reflex to touching; <sup>5</sup>Anesthesia duration: It started at the time of transferring fish from anesthetic solution to a basin with clean water (without drug) it was finished while the first muscle movement (especially tail movement) appeared in fish; <sup>6</sup>The elapsed time from the introduction of fish to clean water (basin contains fresh-water without drug) to time that fish swam normally and strongly was recorded; <sup>7</sup>The elapsed time from the introduction of fish to drug water (basin contains water and the drug) to full recovery time when fish started swimming normally and strongly was recorded.

**Table 2:** Identified recovery stages<sup>1</sup> in the fish.

Stages	Activity
I	Effect of anesthetic was going to decrease, and the first movement such as tail movement (or movement of operculum of gill) was observed in fish while it settled at the bottom of basin.
II	Fish started swimming but did not have complete balance. It tried to go away from touching but could not swim strongly. Fish swam to the left or right side.
III	Fish had normal position and could swim rapidly and strongly with complete equilibrium.

<sup>1</sup>Based on observation of present research.

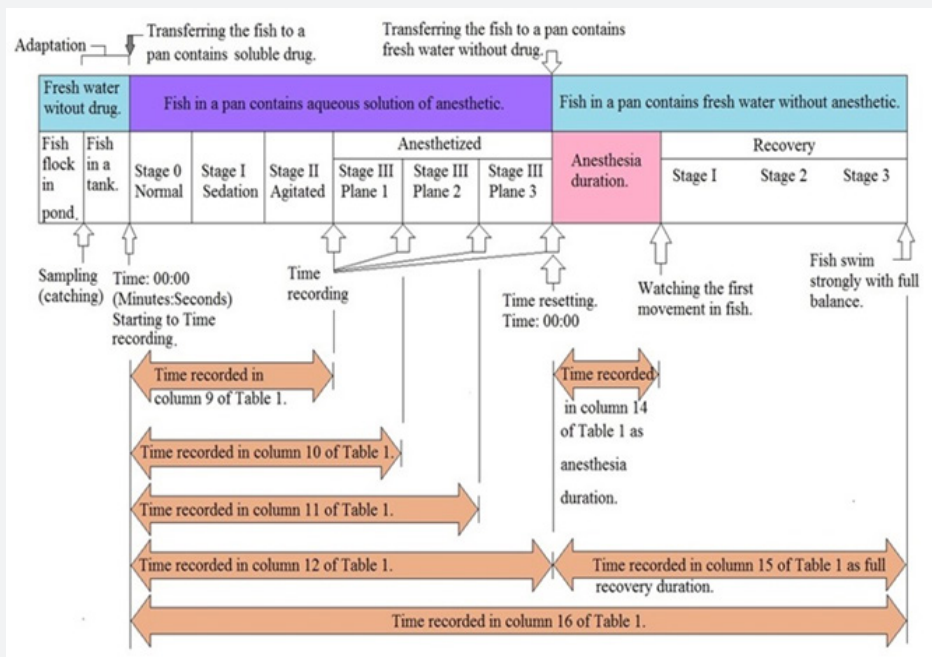


Figure 2: Chart of recorded times.

### Anesthesia levels

Anesthesia in fish has different stages such as I, II, III and IV. Stage III may have 3 planes (plane 1, 2 and 3) [18].

### Ethical reason

In aquaculture, artificial insemination applies for the reproduction of the fish (Rainbow trout). Based on this, the abdominal region of the brood stocks is examined and the male and female brood stocks which can produce sperm and eggs are separated from the fish flock. Then, sperm and egg are extracted from the fish by pressing the chest area to the tail by hand. This operation is not pleasant for alert fish. Therefore, the use of anesthesia can greatly reduce the pain and discomfort of the fish. Accordingly, anesthetics studies not only promote the development of pharmacology for aquaculture but also may provide a suitable anesthetic to reduce stress and pain during artificial reproduction in fish. On the other hand, anesthetics help to reduce stress and pain in fish during handling, transferring, artificial reproduction and clinical procedures. Therefore, there were important reasons for conducting this research, so this research is not unethical. On the other hand, the sample was very small (samples were at least). Each treatment contained only 10 fish as a sample, while generally 30 fish is needed for statistical analysis. If less than 10 fish were used, the results would be unreliable. Adaptation time was also considered for the sampled fish to be more comfortable.

### Statistical method

SPSS software was applied for statistical analysis. In Table 1, data of column no: 14 were analyzed statistically. Data analysis

was done by One-way ANOVA test (Multiple comparisons were done by Tukey & LSD).

### Results

It was determined that Ketamine can cause anesthesia in rainbow trout as dissolved ( $100 \text{ mgL}^{-1}$ ) in water, so it is an effective anesthetic in the fish because all fish (%100 of samples) had been anesthetized. It was a safe drug because all fish (%100 of samples) were alive after the test. Even, the mortality rate was not recorded until 2 hours after the test. The results show that the drug was probably absorbed through gills of fish and it was transferred by the blood circulation to fish body and made anesthesia in fish by the effect on CNS. Four groups (treatments) were tested; they had different mean weight (Table 1). It was found that recovery had 3 different stages in the fish (Table 2). Results show that ketamine ( $100 \text{ mgL}^{-1}$ ) caused anesthesia in fish with weight 22, 51, 69 and 243 grams after 110, 140, 180 and 190 seconds, respectively. Recovery was observed after 537, 512, 620 and 757 seconds, respectively.

In summary, Ketamine solution (100 ppm) in water caused anesthesia in fish after 2-3 minutes approximately, and its effect remained for 5-6 minutes while fish was out of reach of the drug. Fish were recovered to normal condition after 10-16 minutes approximately. However, it is necessary to arrange an international standard protocol for evaluating the effects of different anesthetics in every fish species. Mean value of anesthesia duration included  $370.3 \pm 10$  seconds for treatment-1,  $329.6 \pm 22$  sec for treatment-2,  $300.0 \pm 12$  sec for treatment-3 and  $280.0 \pm 5$  sec for treatment -4. The mean value of anesthesia duration for each treatment was

different in the compared to other treatments ( $p < 0.05$ ) based on the ANOVA test.

### Discussion

Before DATA comparison, it needs to remember that the confirmed results of this research consist of:

- a. Ketamine can cause anesthesia in fish as a soluble form in water.
- b. Applied dose was 100 PPM for fish size between 20-240g.

- c. It is a safe drug for the fish (*Oncorhynchus mykiss*) without mortality.

However, there are some research about the effects of Ketamine. In 2016, it was claimed that Ketamine as 1250, 2500, 3750 and 5000 mg/L concentrations can cause anesthesia on fish (*Acipenser persicus*: Persian sturgeon). It was claimed that “anesthesia induction time” and recovery time of Ketamine in different concentrations for the fish (*A. persicus*) were based on Table 3. They did test for juvenile size of the fish but did not introduce the best concentration of Ketamine in the fish [28].

**Table 3:** Anesthesia induction time and recovery time of Ketamine in different concentrations for the fish (*A. persicus*).

Anesthetic drug (Ketamine) concentration (mg/L).	Time to anesthesia (min)	Time to recovery (min)	Survival (%)
1250	12.01±0.9	9.63±0.60	100
2500	10.27±1.08	8.97±0.94	100
3750	8.36±0.48	7.54±0.82	100
5000	7.08±1.14	9.06±0.56	100

Mohammadi & Khara [29] applied Ketamine as 0.5-1.7 mg/L to induction anesthesia in juvenile fish (*O. mykiss*). The results of the present work suggest that Ketamine as 100 mg/L concentrations can cause anesthesia on fish (*O. mykiss*; Size: 20-240g). The recent case shows a concentration 10 times lesser than research of Adel & et al [28]. They mentioned that 500mg/L of Ketamine could not cause anesthesia in the fish (*A. persicus*). It may show that (*O. mykiss*) is more sensitive to Ketamine than (*A. persicus*) because 100 mg/L concentration of Ketamine made anesthesia in Rainbow trout while the fish (*A. persicus*) have normal behavior in 500mg/L of Ketamine.

However, number of survival fish of the two research is equal. It shows that Ketamine is a safe drug for two species of the fish even Ketamine as 5g/L concentration didn't cause mortality on fish (*A. persicus*), but such concentration didn't test on fish (*O. mykiss*) in the present work. We studied one concentration of the drug on different size (4-size) of the fish it is shown in Table 1 while Adel studied different concentration of the drug on juvenile fish only. In addition, Adel & et al [28] reported that recovery time did not increase with an increase in anesthetic concentration with Ketamine. They could not explain why recovery time was not related to high and low concentrations of Ketamine.

It was claimed that Ketamine alone provides effective short-term immobilization in the white sturgeon (*Acipenser transmontanus*), leopard shark (*Triakis semifasciata*) and spiny dogfish (*Squalus acanthias*). It has been efficiently used to anesthetize fish when injected in mixture with medetomidine and magnesium oxide [30,31]. We found that Ketamine not only can cause anesthesia in fish without mixing by another drug, but also it is able to cause anesthesia in fish as a soluble form without

injection procedure. We propose that Ketamine could be used as an anesthetic-drug when other mixtures are not available, or injection is not possible. However, Ketamine is a broad-spectrum anesthetic in fish and mammals which can cause immobilization in different species of fishes such as *Salmo trutta*, *Salmo gairdneri*, Cichlids, Elasmobranchs and *Cyprinus carpio* [30,32,33]. Moreover, China recently forbade the use of ketamine and because they are the major producer in the world, its accessibility on the market is probably going to decrease [28]. However, Ketamine is a medical drug and can cause anesthesia in human so its common use may need some limitations [27].

### Conclusion

In man, Ketamine has some unique effects such as profound analgesia, stimulation of the sympathetic nervous system, bronchodilation, and minimal respiratory depression. These effects make ketamine an important alternative to the other intravenous anesthetics and a desirable adjunct in many cases despite the unpleasant psychotomimetic effects. Moreover, ketamine can be administered in multiple routes (intravenous, intramuscular, oral, rectal, and epidural) [27]. Based on the findings of this research, in addition to the above-mentioned properties, Ketamine can cause anesthesia in fish (*Oncorhynchus mykiss*) by gill route as a soluble drug in water. Suitable concentration to do this is about 100 ppm (100 mgL<sup>-1</sup>). Less- or higher concentrations were not tested by this research. On the other hand, its routine application in aquaculture is limited by the high price of Ketamine. However, Ketamine is a choice for safe anesthesia in fish. Its application is easy as it is water-soluble. Therefore, Ketamine is a potent drug that can cause anesthesia in fish (rainbow trout, 18 to 257 g) as 1g/10L and in aqueous form.



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