Research Article

The effects of *Datura stramonium* L. seed extract on anesthesia of farmed *Cyprinus carpio* in Guilan province

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Abstract

The anesthetic effect of Datura seed extract was evaluated on 15 pieces of young *Cyprinus carpio* weighing approximately 25 to 50 g. In order to determine the effectiveness of L. *Datura stramonium* extract on the importance and severity of *Cyprinus carpio* anesthesia in doses between zero mg to 10 mg /L, 10 treatments (zero treatment, control) were devised with the identical conditions (pH 7/2, temperature 22 °C, extract and fish). When fish were placed in different treatments in the drug bath, the onset time of anesthesia, the time of complete anesthesia, deep anesthesia and mortality percentage were measured by a chronometer. The results showed that increasing the concentration of Datura extract reduced the onset time of anesthesia. There was a significant difference between the onset time of anesthesia in the range between 6 mg /L and 8 mg / L ($p \leq 0.05$), There was no significant difference between 6 and 7 mg / L and 7 and 8 mg / L ($p > 0/05$). There was also a significant difference between the mean time of complete anesthesia between 6 and 8 mg / L treatments ($p \leq 0.05$) but there is no significant difference between 6 and 7 mg / L and 7 and 8 mg / L ($p > 0/05$). With increasing drug concentration, the mean return time from anesthesia in the treatments was different and a significant difference was observed between 6 and 8 mg / L ($p \leq 0.05$).

Key words: *Datura stramonium,* Extract, Anesthesia, *Cyprinus carpio*

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Introduction

Datura plant (*Datura stramonium* L.) belongs to the Solanaceae family. It is an annual

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herbaceous plant (30 - 80 cm in height) characterized by branched stems and broad leaves, easily distinguishable from afar by its special appearance. Datura has mainly originated in India and the Caspian Sea coast, but today it is distributed in most parts of the United States, Europe, Asia and Iran (Devi, *et al*., 2011; Gaire and Subedi, 2013). Datura has relatively thick roots with round, branched stems. Its broad, pointed leaves are 10 to 15 cm long and 7 to 10 cm wide. There are 5 to 7 identifiably pointed and toothed lobes in them that are irregular in shape (Devi, *et al*., 2011). Since early in life, humans use plants for various purposes, such as food and medicine. Many people still use different herbs to treat various ailments. *Datura stramonium* is the most important medicinal plant in traditional medicine due to its many properties (Khan, *et al*., 2013). Datura contains a variety of phyto chemicals including saponins, tannins, steroids, alkaloids, flavonoids, phenols and glycosides (Shagal, *et al*., 2012). It's important alkaloids are atropine, hyosin and hyoscyamine. The amount of alkaloids in the plant leaves is less than that of in seeds (Jakabová, 2012). In traditional medicine, it was used to treat many diseases, by mixing the leaves with mustard oil in the treatment of dermal disorders. The leaf extract is used to relieve pain, while the seeds are used for coughs and fevers. The leaf extracts are also applied in the treatment of wounds, hemorrhages, pain, baldness, parasitic infection and muscle spasms (Preissel and Preissel, 2011; Diker *et al*., 2007; Das, *et al*., 2012). In modern medicine, the plant is used for its medicinal effects as an antispasmodic and/or bronchodilator agent due to the anticholinergic properties of atropine and scopolamine, which act by inhibiting the muscarinic receptor (M2) on the smooth muscles of the bronchi and submucosal gland cells (Peredery and Persinger, 2004; Charpin, *et al*., 1979). They use the anti-cholinergic properties of Datura plant to neutralize organophosphate poisonings. Experiments showed their efficacy to eliminate acute poisoning in mice. It has also proved effective in alleviating epilepsy in mice (Bania *et al*., 2004). The methanolic extract of the plant showed inhibitory properties on gram-positive bacteria such as Vibrio, *Staphylococcus aureus*, and on *Klebsiella bacteria* (Eftekhar, *et al*., 2005; Shagal, *et al*., 2012). The Estonian extract of this plant has antifungal properties on *Aspergillus niger*, *Aspergillus parasiticus*, *Penicillium expansum* and *Trichoderma harziamum* (Mdee, *et al*., 2009). Datura ethanolic extract has inhibitory properties on edema and inflammation of rat paw (Sonika, *et al*., 2010). For research activities on fishes as in biometrics, blood sampling, surgery, transfer, periodic sampling, and artificial reproduction, it is necessary to anesthetize fish so as to reduce pain, stress, and prevent disturbances in physiological activity. Due to the lack of herbal sedatives and anesthetics in fish, suitable alternatives that are readily available should be provided instead of various chemical compounds such as methomidate, phenoxyethanol, benzocaine, metric sulphate triacine (MS222) (Husen and Sharma, 2014). *Cyprinus carpio* farming is of great economic importance in the country today. This fish species is propagated every year in hatcheries

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of both public and private sectors. One of the threats in fish handling is transport stress, which causes losses due to physiological, intense metabolic and ionic disorders (Mazandarani,*et al*.,1998).. It is therefore, crucial to have a relaxed fish handling procedure via materials that could control and reduce losses during their transportation, sampling and artificial reproduction (Rahanandeh, 2021). Due to its different alkaloid compounds and varied sedative, analgesia and anti-inflammatory effects, Datura seed extract has various applications in medicine making it a good alternative to other anesthetic chemical compounds in fish.

Materials and methods

This study was conducted from April to June 2021 in Mirzakoochak Khan Guilan Fisheries Science Training Center. To obtain Datura seed extract, 600 grams seeds of this plant were ground into a powder. The powder was soaked in 500 ^{cc} of sterile distilled water for 24 hours, and then heated for 8 hours in a Hot Plate Magnetic Stirrers to mix well. The solution was concentrated after passing through a filter paper using a rotary evaporator connected to the pump by distillation in a rotating vacuum at 50 $^{\circ}$C (Rezvani, *et al*., 2011). To remove excess water, the solution was placed in the freeze drying machine for 3 hours to dry and a pure extract was prepared, in which case 15 g of the solution was prepared. Then 500 mg of this solution was dissolved in 250 mL of sterile distilled water to prepare 250 mL of stock solution. In this case, each mL of stock obtained, contained 20 mL of Datura extract (Rezvani, *et al*., 2011). In order to determine the effectiveness of L. *Datura stramonium* extract on anesthesia of carp in the amount of between zero mg / l to 10 mg / L in 10 treatments (zero control treatment) with the same conditions (temperature 24 °C, pH 7.2) under were affected by this drug. The duration of fish stay in the drug bath was calculated in different treatments from the onset of anesthesia, using plateau stopwatch (Model f-011) including the time measurement of complete anesthesia, deep anesthesia and the percentage of losses. At the time of preliminary testing, water inlet and outlet and water hydrochemistry were considered constant. After the preliminary test, it was found that application of 6, 7 and 8 mg / L of the drug could bring carp to complete anesthesia without causing death. The results of the preliminary test are shown in (Table 1).

Table 1. Preliminary testing in 10 different treatments of *Datura stramonium* L. extract

Drug concentration (mg / L)→	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Superficial anesthesia	−	−	−	−	+	+	+	+	+	+	+
Complete anesthesia	−	−	−	−	−	−	+	+	+	+	+
Deep anesthesia	−	−	−	−	−	−	−	−	−	+	+
Fatality	−	−	−	−	−	−	−	−	−	+	+

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After selecting the effective values in anesthesia based on the preliminary experiment, 3 concentration types of *Datura stramonium L*. extract were selected to compare the time of anesthesia. Then 4 treatments including control and values of 6, 7 and 8 mg / L and 3 replications of each treatment were considered. In order to measure the time of entry of superficial anesthesia, complete anesthesia and deep anesthesia, 10 pieces of young carp were designated in each replication and the desired times were measured. The percentage of losses at the end of the experiment was recorded in each replicate. During this experiment, the physicochemical factors of water were similar to the preliminary experiment (pH 7/2, temperature 22 ˚C) (Altun, *et al*., 2006; Padiyoor, *et al*., 2017; Velisek, *et al*., 2005).

Statistical method

Data were analyzed in Spss20 software after execution in Excel software. Before examining the data, their normality was checked using a Kolmograph-Smirnov test. One-way analysis of variance was used to determine the statistically significant difference between the studied treatments and Duncan's test was used to separate the studied groups. Data were presented based on mean and standard deviation.

Results

The results of the effect of L. datura stramonium extract on anesthesia among young carp are presented in Table 2 and Figures 1 to 3. As mentioned earlier in the working method; treatments of 1 to 3 mg /L had no effect on fish anesthesia. In treatments, 4 and 5 mg / L induced superficial anesthesia in young fish after 40 minutes. In 6 mg / L treatment and in 7 mg treatment, complete anesthesia occurred after 30 minutes and 20 minutes respectively while in 8 mg / L treatment, anesthesia occurred after 10 minutes**.** At 9 mg / L treatment, complete anesthesia was induced after 10 minutes and they entered the deep anesthesia stage after 20 minutes. Deep anesthesia was performed on fish at 10 mg / L after 10 minutes. At 9 and 10 mg / L, after deep anesthesia, gills were damaged and fish died. In treatments of 6, 7 and 8 mg / L after superficial anesthesia, no instances of fish mortality or fish gill damage were observed when young fish entered the stage of complete anesthesia, and the return time from anesthesia was associated with no problem. The results showed that with increasing drug concentration, the duration of onset of anesthesia had a decreasing trend. Also, the duration of complete anesthesia was shorter and the return time was longer than anesthesia (Figures 1, 2, 3). The results of a comparative experiment to determine the onset times of anesthesia showed a significant difference in the entry of fish into the stage of superficial anesthesia ($p \leq 0.05$) (Table 2). These results showed that fish at 7 and 8 mg / L concentrations enter the stage of superficial anesthesia faster than the concentration of 6 mg / L ($p \leq 0.05$). The fastest time for fish to enter the stage of complete anesthesia occurred at the treatment of 8 mg / L (Table 2) and this time was not statistically significant with the treatment of 7 mg / L ($p > 0.05$). The mean time

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for fish to enter the stage of complete anesthesia was longer at 6 mg / l showing a significant difference with those of other two treatments ($p \leq 0.05$) (Table 2). Therefore, proportional to the concentration increase of anesthetic in the treatments, the onset time of anesthesia had a decreasing trend and the return time from anesthesia was longer. The mean time of fish entering superficial and complete anesthesia in the 6 mg / L treatment was longer compared to 8 mg / L but the mean time of fish returning from complete anesthesia was shorter than the 8 mg / L treatment ($p \leq 0.05$) (Table 2). The mean return time from complete anesthesia was not significantly different between treatments of 6 and 7 mg /L ($p > 0.05$). The mean return time from anesthesia did not show a significant difference between 7 and 8 mg / L ($p > 0.05$) (Table 2).

Table 2. Mean (± standard deviation) onset time of anesthesia, complete anesthesia, return time from anesthesia and percentage of mortality of *Datura stramonium L* extract

Treatments	Mean time to start anesthesia (seconds)	Mean time of complete anesthesia (seconds)	Mean return time from anesthesia (seconds)	Percentageof fatality
Control	0	0	0	0
6 mg / L	510.00 ±52.6^{b}	1050.00±134.16^{b}	623.33±168.15^{a}	0
7 mg / L	468.33 ±51.54^{a}	410.00±16.37^{a}	903.33±170.15a^{b}	0
8 mg / L	350.00 ±63.35^{a}	343.32±43.20^{a}	1125.83±191.52^{b}	0

* The different letters of the tablets indicate a significant difference in different concentrations of the drug

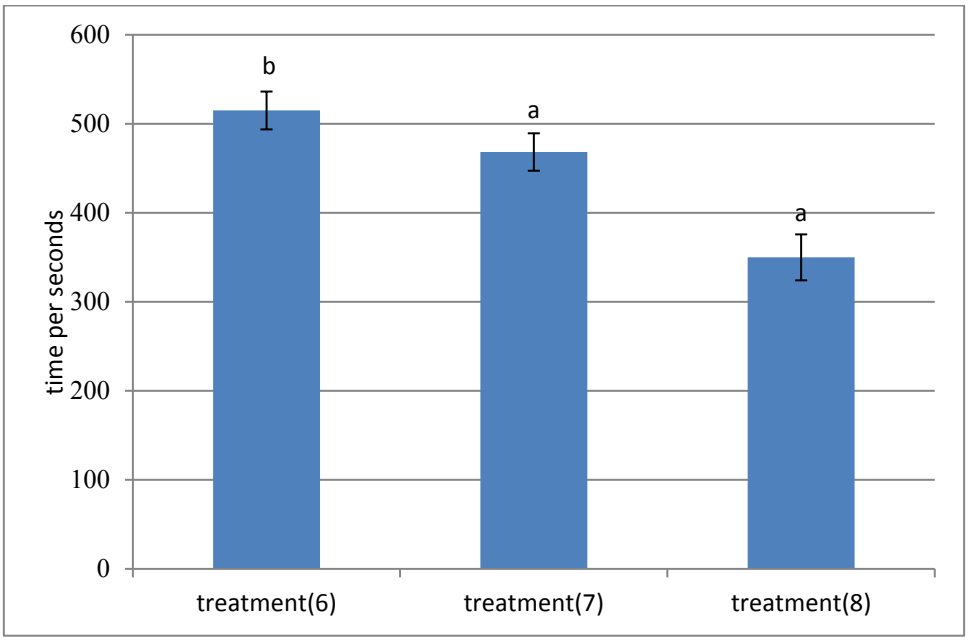

Figure 1. Mean time of onset of anesthesia at different concentrations of Datura extract in young *Cyprinus carpio*.

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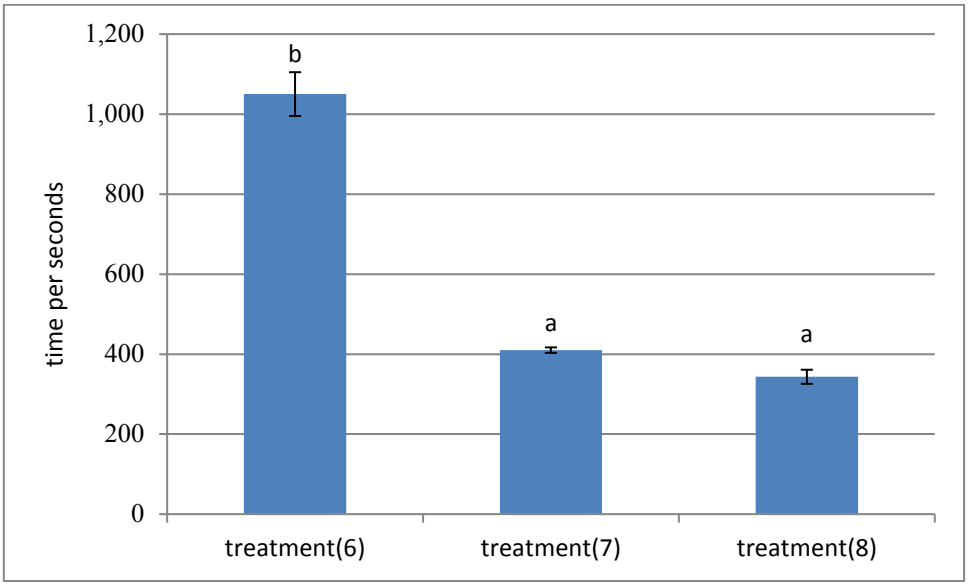

Figure 2. Mean time of complete anesthesia at different concentrations of Datura extract in young *Cyprinus carpio* p.

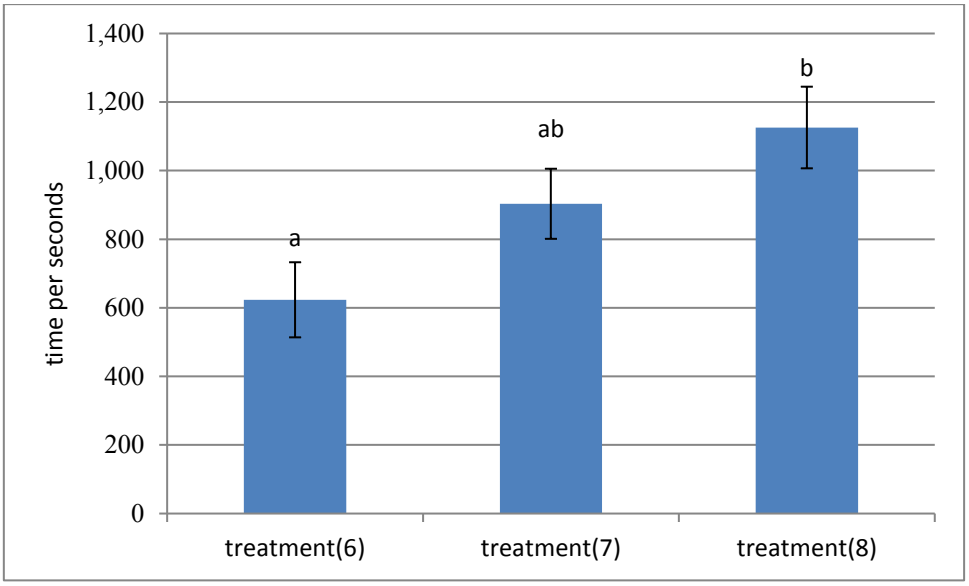

Figure 3. Mean return time from anesthesia at different concentrations of Datura extract in young *Cyprinus carpio*.

Discussion

The human use of *Datura stramonium L.* seed extract is for various therapeutic purposes such as analgesia, sedation, anticonvulsant, wound inflammation, etc. (Zargari, 1997) and (Kirtikar and Baso, 1999; Gaire and Subedi, 2013; Sayyed and Shah ,2014). Various drugs are used in aquatic animals to induce sedation and anesthesia, such as tricaine, methane sulfate (MS222), clove extract), benzocaine, phenoxyethanol and each of these substances has different efficacy as per concentration, sedation and anesthesia. (Sneddon, 2012; Husen and Sharma, 2014). (Soltani, 2007) reported the effect of clove extract on carp

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anesthesia and it was announced that by increasing the concentration of clove extract, the onset time of anesthesia decreases. In another study, (Mortazavi zadeh, *et al*., 2012) reported the effect of propofol concentration on *Barbus sharpeyi* maintaining that it can cause anesthesia in fish. The time of onset of anesthesia in fish decreased with increased concentration of the drug. The results of this study also showed that the mean duration of anesthesia at different concentrations of the drug was different in young carp fish and the difference was significant between 6 and 8 mg / l ($p\leq0.05$). Also, when the concentration of Datura extract increased, the mean onset duration of anesthesia showed a decreasing trend. (Table 2 and Figures 1 to 3). (Gomes, *et al*., 2001), when investigating the effect of benzocaine concentrations for anesthesia on *Colossoma macropomum*, reported that with increasing benzocaine concentrations, the average time of onset of anesthesia in fish decreased. Complete anesthesia and return time from anesthesia varied at different concentrations, and when the drug concentration increased, the mean time from complete anesthesia and return time from anesthesia was longer. The results of the above researchers were consistent with the findings of this study. In a study on the effects of different amounts of propofol on complete anesthesia in *Barbus sharpeyi* fish and also the return time from anesthesia in different amounts of propofol Mortazavi zadeh, *et al*., (2012) stated that the average time of complete anesthesia and return time from anesthesia at different concentrations of propofol in fish Bani has been meaningful. (Keene, *et al*., 1998) reported the effects of different concentrations of clove oil on salmon. The time of onset of anesthesia, complete anesthesia, and the time of return from anesthesia tended to increase as the drug concentration rose because the drug concentrations were not completely eliminated from the body and the difference was significant. (Soltani, 2007) reported a similar report on the effects of Clove extract and essence on carp and sturgeon. In this study, when the concentration of the extract increased from 6 mg /L to 7.8, 9 mg / L, the mean duration of anesthesia decreased and the duration of complete anesthesia and the time of return from anesthesia to higher concentrations were longer. This difference between concentrations 6 and 7 and between 7 and 8 was not significant ($p>0.05$). However, between 6 and 8 this difference was significant and the results of this study showed that The mean time of onset of anesthesia, complete anesthesia, and return time of anesthesia are consistent with other researchers' findings on benzocaine, clove extract, rosehip oil, propofol, and vujinol. The results of this study showed that the onset time of anesthesia, complete anesthesia and the time of return from anesthesia are consistent with the findings of other researchers on benzocaine, clove extract, migraine oil, propofol and vujinol. The conclusion is that throughout different research works, it was revealed that all 3 concentrations of Datura 6, 7, and 8 mg / L could be used to induce anesthesia in fish. However, it is not recommended to use 9 and 10 mg / L concentrations because of the ensuing young fish mortality caused by deep anesthesia.

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Conflict of interest

Authors have no conflict of interest on this work.

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