Short Communication

Anesthetic efficacy of ketamine–diazepam, ketamine–xylazine, and ketamine–acepromazine in Caspian Pond turtles (Mauremys caspica)

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Abstract:

Objectives: The objective of this study was to assess the efficacy of different anesthetic drug combinations on the Caspian Pond turtles (Mauremys caspica).

Subjects and Methods: Three groups of the Caspian Pond turtles (n = 6) were anesthetized with three different drug combinations. Initially, a pilot study was conducted to determine the best drug doses for the anesthetization of the turtles, and according to these results, ketamine–diazepam (120 mg/kg ketamine hydrochloride [5%] and 2 mg/kg diazepam [5%]), ketamine–acepromazine (120 mg/kg ketamine hydrochloride [5%] and 1 mg/kg acepromazine [1%]), and ketamine–xylazine (120 mg/kg ketamine hydrochloride [5%] and 1 mg/kg xylazine [2%]) were injected intramuscularly. The onset times of anesthetization and the recovery time were measured. Statistical analysis of the data was performed using one-way analysis of variance followed by t-tests, and P < 0.05 was considered statistically significant.

Results: There were statistically significant differences in the mean of the onset times of anesthesia and recovery time among the three drug combinations depending on the treatment used. The onset of anesthesia of the animals treated with the ketamine–diazepam combination was 60% and 42% shorter, for male and female turtles, respectively, compared to that obtained with the ketamine–acepromazine combination and 64% (male turtles) and 50% (female turtles) shorter than that obtained with the ketamine–xylazine combination. Further, the recovery time, in male turtles, was 17% shorter in animals treated with the first drug combination than those treated with the ketamine–acepromazine combination and 37% shorter than those treated with the ketamine–xylazine combination. The recovery time, in female turtles, did not seem to be significantly different among treatments.

Conclusions: The study showed that the ketamine–diazepam drug combination is the anesthetic combination with the fastest onset time and shortest recovery time.

Key words: Anesthetics, diazepam, ketamine, Mauremys caspica

Anesthetic and analgesic drugs, such as ketamine, diazepam, acepromazine, and xylazine, are utilized in freshwater and sea turtles to reduce stress and prevent mortality during transportation, handling, examination, manipulation, and surgical procedures.[¹] Ketamine, midazolam, diazepam, and propofol are all used as turtle anesthetics and analgesics to some degree and with varying efficiency. Ketamine, in particular, is a widely diffuse anesthetic and is used in combination with other injectable agents such as benzodiazepines, α₂-agonists, or propofol. The Caspian Pond turtle (Mauremys caspica) is an important species of turtle whose conservation status has not yet been evaluated. The turtle belongs to the family Geoemydidae and lives in the eastern Mediterranean region, from northwest of Saudi Arabia, Iraq, Bahrain, Turkey, Caucasus, Tbilisi to northern, central, and southwestern part of Iran.[²] This species is also widely dispersed in various provinces of Iran including Mazandaran, Golestan, Guilan, Ardabil, Azerbaijan, Kurdistan, Fars, and Khuzestan.[³] Despite the importance of biodiversity and high frequency of this precious species, no studies have been carried out to investigate the effects of anesthetic agents in the Caspian Pond turtles. The objective of this study

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was to assess, for the first time, the efficacy of anesthetic and analgesic drug combinations, particularly that of ketamine hydrochloride and diazepam, ketamine hydrochloride and acepromazine, ketamine hydrochloride and xylazine, on Caspian Pond turtles, through the measure of two important parameters: The onset time of anesthesia and time taken for the total recovery of the turtle.

**Subjects and Methods**

**Location, Sampling, Animal Care**

Eighteen Caspian Pond turtles (*M. caspica*) were collected from Tajan and Shirod River, in the Mazandaran province, during the months of September and October 2014.

Permission to collect the Caspian Pond turtles was authorized by the Iran Department of Environment (Permission Number: 1184-N/14/2; September 22, 2014). The turtles, whose average age was of 3 ± 1 years, were transported alive to the central laboratory of Caspian Sea Ecology Research Centre, and their total lengths (cm) and total weights (g) were measured. In particular, for male turtles, the average length was 11.3 ± 3.8 cm and the average weight was 277.3 ± 14.2 g. For female turtles, the average length was 10.84 ± 2.6 cm and the average weight was 277.18 ± 10.6 g.

To let the animals adapt to the new condition, turtles were kept in a turtle glass tank, containing river water, for 1 week, and water physicochemical parameters, such as temperature (21.5 ± 1.34°C), pH (7.7 ± 0.3), dissolved oxygen (DO) (6.8 ± 0.7 mg/L), and salinity (3.8 ± 0.4 ppt), were monitored. DO was estimated by Winkler’s method and salinity was measured by an Erma hand refractometer (Tokyo).

**Preparation of Anesthetic Solutions**

Ketamine (5%), acepromazine (1%), and xylazine (2%) were purchased from Alfasan Company. Diazepam (5%) was purchased from Caspian Tamin Company.

Initially, a pilot study was conducted to determine the best dose of these drugs for the anesthetization of the Caspian Pond turtles.

**Experimental Study**

The male and female turtles were divided into three groups of six animals each. Data from the pilot study were utilized to determine the concentration of the anesthetics for each treatment. Therefore, three different drug combinations, 120 mg/kg ketamine hydrochloride (5%) - 2 mg/kg diazepam (5%), 120 mg/kg ketamine hydrochloride (5%) - 1 mg/kg acepromazine (1%), and 120 mg/kg ketamine hydrochloride (5%) - 1 mg/kg xylazine (2%) were injected intramuscularly in the left thoracic member through a 20-gauge needle. The onset of anesthesia, that is the time needed to reach the third stage of anesthesia (target plane of anesthesia), Park JS et al.[12] [Table 1], and the recovery time (from the end of the third stage to the ambulatory recovery) were recorded, using a digital stopwatch, for each treatment individually.

The experiment was performed in triplicate. During the experiments, turtles were kept in fiberglass tank (50 L) and temperature was monitored with a thermometer (21.8 ± 1.4°C). At the end of the study, turtles were set free.

### Table 1: Stages of anesthesia in the Caspian Pond turtle, *Mauremys caspica*

| Stage | Description |
|-------|-------------|
| Stage I | Sedation, partial loss of reaction to external stimuli |
| Stage II | Partial loss of equilibrium, uncoordinated movement followed by active swimming |
| Stage III | Total loss of equilibrium, soft-shelled turtle turns over after blowing air from nose but retains swimming ability |
| Stage IV | Anesthesia, total loss of reflex activity and swimming ability, soft-shelled turtle fails to respond to strong external stimuli. Recovery in anesthetic-free water routine. A desirable level of anesthesia |
| Stage V | Medullary collapse, relax of neck and four legs, a dangerous level of anesthesia that should be avoided |

**Statistical Analysis**

Data were analyzed using the SPSS software version 18 (SPSS Inc., Chicago, IL, USA). Statistical analysis was performed using one-way analysis of variance followed by *t*-tests and *P* < 0.05 was considered statistically significant.

**Results**

**Pilot Study**

Results from a pilot study did not show anesthetic effects utilizing drug combinations of 50–80 mg/kg ketamine hydrochloride (5%) and 0.5–1.3 mg/kg diazepam (5%), 40–70 mg/kg ketamine hydrochloride (5%) and 0.2–0.4 mg/kg acepromazine (1%), and 50–80 mg/kg ketamine hydrochloride (5%) and 0.1–0.3 mg/kg xylazine (2%). Furthermore, the drug combinations such as 70–80 mg/kg ketamine hydrochloride (5%) and 1–2 mg/kg diazepam (5%), 80–90 mg/kg ketamine hydrochloride (5%) and 0.5–0.7 mg/kg acepromazine (1%), and 80–100 mg/kg ketamine hydrochloride (5%) and 0.5–0.7 mg/kg xylazine (2%) let the turtles reach the Stage II of anesthesia.

In addition, with 180 mg/kg ketamine hydrochloride (5%) and 3 mg/kg diazepam (5%), 200 mg/kg ketamine hydrochloride (5%) and 2 mg/kg acepromazine (1%), and 150 mg/kg ketamine hydrochloride (5%) and 1.5 mg/kg xylazine (2%), turtles reached the Stage V of anesthesia [Table 1], and dangerous levels of anesthesia were observed. Furthermore, by increasing the different drug combinations, the onset time of anesthesia decreased and the recovery time increased.

**Anesthetic Efficacy of Ketamine–Diazepam, Ketamine–Xylazine, and Ketamine–Acepromazine**

To perform the experiments, 18 Caspian Pond turtles were divided into three groups and treated with the anesthetic drug combinations.

Animals were kept in a turtle tank for a week, and water physicochemical parameters, such as temperature, pH, DO, and salinity, were monitored.

According to the results from the pilot study, the drug combinations of 120 mg/kg ketamine hydrochloride (5%) and 2 mg/kg diazepam (5%), 120 mg/kg ketamine hydrochloride (5%) and 1 mg/kg acepromazine (1%), and...
120 mg/kg ketamine hydrochloride (5%) and 1 mg/kg xylazine (2%) were administered. Data analysis showed that there are statistically significant differences in the mean of the onset time of anesthesia among the three combination drugs [Figure 1]. In particular, for male turtles, the onset of anesthesia of the animals treated with the ketamine–diazepam combination (29.16 ± 2.64 min) was 60% shorter (P < 0.0001) compared to that obtained with the ketamine–acepromazine combination (73.67 ± 5.23 min) and 64% shorter (P < 0.0001) than that obtained with the ketamine–xylazine combination (80.25 ± 3.16 min). In female turtles, the onset time of anesthesia was 42% shorter (P = 0.0017) in animals treated with the ketamine–diazepam combination (37.33 ± 3.28 min) than those treated with the ketamine–acepromazine combination (51.16 ± 4.37 min) and 37% shorter (P < 0.0001) than those treated with the ketamine–xylazine combination (67.7 ± 2.18 min).

The recovery time, in male turtles, was 17% shorter (P < 0.0001) in animals treated with the first drug combination (42.33 ± 2.60 min) than those treated with the ketamine–acepromazine combination (51.16 ± 4.37 min) and 37% shorter (P < 0.0001) than those treated with the ketamine–xylazine combination (67.7 ± 2.18 min).

The recovery time of ketamine hydrochloride–diazepam combination, in female turtles, did not seem to differ significantly from ketamine–acepromazine (P = 0.49) or ketamine–xylazine combinations (P = 0.06). These results showed that the time measurements relative to the different drug combinations [Table 2] were in accordance with the following sequence: Ketamine–diazepam < ketamine–acepromazine < ketamine–xylazine. Furthermore, the recovery time, following the induction of anesthesia by ketamine–diazepam, was also significantly faster than ketamine–acepromazine or ketamine–xylazine combinations.

Table 2: Onset of anesthesia and recovery time induced by the anesthetic agents in *Mauremys caspica* male and female turtles (mean ± standard deviation)

| Anesthetic agent | Parameter (min) | Male | Female |
|------------------|----------------|------|--------|
| Ketamine-diazepam| Onset of anesthesia | 29.16±2.64a | 37.33±3.28a |
| Recovery time    | 42.33±2.60a     | 52.67±3.18a |
| Ketamine-acepromazine | Onset of anesthesia | 73.67±5.23a | 64.67±2.72a |
| Recovery time    | 51.16±4.37a     | 50.33±1.20a |
| Ketamine-xylazine| Onset of anesthesia | 80.25±3.16a | 74.33±2.33a |
| Recovery time    | 67.7±2.18a      | 58.12±2.30a |

Different alphabetic superscripts in the same row indicate significant differences between the compared groups (P<0.05)

Discussion

A safe and effective anesthetic protocol for the care of turtles, and in particular of the Caspian Pond turtles, is really important, and unfortunately, there are not enough studies on the clinical anesthetic effects of drug combinations in these animals.

There have been various studies that have evaluated the efficacy and side effects of different drug combinations, such as propofol and ketamine in Giant South American turtle *Podocnemis expansa*, midazolam and propofol in black-bellied slider *Trachemys orbignyi*, lidocaine hydrochloride and sodium bicarbonate in soft-shelled turtle *Pelodiscus sinensis*, medetomidine and ketamine in gopher tortoises *Gopherus polyphemus*, and ketamine and midazolam in Snapping turtles *Chelydra serpentina*.

Ketamine hydrochloride is an anesthetic and analgesic drug that has been widely used both in human and veterinary medicine, especially in fishes and tortoises, and the appropriate dose of the drug for the anesthetization of these animals, such as grass carp *Ctenopharyngodon idella*, Giant South American turtle *P. expansa*, gopher tortoises *G. polyphemus*, snapping turtles, and the Central Asian tortoise *Testudo horsfieldii*, is well documented in literature.

Xylazine can be used in conjunction with local anesthetics. In particular, the efficacy of xylazine in combination with ketamine and midazolam, in Central Asian tortoise *T. horsfieldii*, or in combination with ketamine, in the common carp *Cyprinus carpio* and grey nurse sharks *Carcharias taurus*, has been widely evaluated.

Acepromazine is one of the phenothiazine derivatives with long-acting sedative effect that is mostly commonly used in small animals. This drug has a strong sedative effect, long
recovery time, weak analgesic effects,\textsuperscript{[13]} and has limited use in aquatic animals. In fact, there is only one study in which the dose-response curve of acepromazine, in Giant South American turtles \textit{P. expansa}, has been evaluated.\textsuperscript{[14]} The \textit{M. caspica} (Gmelin, 1774) is a medium-sized freshwater turtle that is widespread throughout the Middle East. In Iran, the Caspian Pond turtle is widely distributed in the north, west, and southwest of the country.\textsuperscript{[17‑19]} While the species is still common in many of these areas, landscape alteration, pollution, and intensification of water management in Turkey, Syria, Iraq, and Iran are an increasing threat to the survival of these animals.\textsuperscript{[20]} Worldwide, populations of wild freshwater and sea turtles are declining at an alarming rate, and the veterinary community can play an important role in conservation by participating in efforts to provide medical care to these animals. The importance of anesthetic and analgesic drugs for the animal medical cares has previously been described,\textsuperscript{[2,3]} such as the importance of the studies about the efficacy and efficiency of the drugs on the animals. To select the best anesthetic protocol, for any patient, the key elements are efficacy, safety, and reliability. An effective protocol provides for a rapid and smooth anesthesia induction as well as a fast and optimal recovery. Furthermore, the optimal anesthetic agent has a rapid onset of action and is quickly cleared from the bloodstream and central nervous system. The ideal drug does not affect the circulatory system and vital tissues or cause adverse effects and is inexpensive. Among different veterinary anesthetic drugs, we focused our attention on three different combinations of the widely used anesthetics and sedatives: Ketamine, diazepam, acepromazine, and xylazine. Ketamine hydrochloride is a general anesthetic agent characterized by analgesia, rapid induction, and limited duration of action. The analgesic action of ketamine is due to its ability to bind the N-methyl-D-aspartate subtype of glutamate receptor. Unfortunately, ketamine has significant side effects mainly due to its effects on the cardiovascular system. The association of ketamine with diazepam, a sedative with anti-anxiety, muscle-relaxing, and hypnotic properties, not only can reduce side effects of ketamine but also can prolong and enhance its anesthetic effects.\textsuperscript{[21]} Acepromazine is a veterinary drug used as a sedative and preanesthetic compound. It is a dopamine antagonist that inhibits the binding of the neurotransmitter dopamine to D2 receptors. Xylazine is an injectable veterinary drug that belongs to the alpha-2-adrenergic agonists and induces sedation, analgesia, and myorelaxation.

In our experiments, we demonstrated, for the first time, on the Caspian Pond turtles that the time needed to reach the Stage III of anesthesia [Figure 1], using ketamine hydrochloride and diazepam drug combination, was significantly lower than that measured using the other drug combinations.

Furthermore, the measurements of the recovery time followed the same trend [Figure 2]. In fact, as summarized in Table 2, the onsets of anesthesia, for male and female turtles, were found to be, respectively, 40% and 42% faster in animals treated with the ketamine hydrochloride and diazepam combination than those treated with the ketamine hydrochloride and acepromazine combination, and 64% and 50% faster than those treated with the ketamine hydrochloride and xylazine combination. The male turtles’ recovery time was 17% shorter than those treated with the ketamine hydrochloride and acepromazine combination, and 64% and 50% faster than those treated with the ketamine hydrochloride and acepromazine combination and the ketamine hydrochloride and xylazine combination. The female turtles’ recovery time was 37% shorter than those treated with the ketamine hydrochloride and xylazine combination. The female turtles’ recovery time does not seem to be significantly different among treatments. These results led us to conclude that among the three drug combinations analyzed, the ketamine hydrochloride and diazepam combination is the best to fulfill the criteria of a favorable anesthetic. In fact, the treatment produces, among the drug combinations tested, the faster effects in terms of the onset of anesthesia and recovery. There was no evidence that the measurements of efficacy differed between male and female turtles with any of the drug combinations.

![Figure 2: Anesthesia recovery time in male and female turtles according to the anesthetic drug combinations (*P < 0.001). Recovery time in male (red bar) and female (blue bar) turtles postanesthetic administration of the following drug combinations: 120 mg/kg ketamine hydrochloride (5%) and 2 mg/kg diazepam (5%), 120 mg/kg ketamine hydrochloride (5%) and 1 mg/kg acepromazine (1%), and 120 mg/kg ketamine hydrochloride (5%) and 1 mg/kg xylazine (2%). Statistical analysis was performed using one-way analysis of variance followed by t-tests (*P < 0.001)](image-url)

Conclusions

The Caspian Pond turtle is an important species of turtles, whose conservation status has not yet been evaluated that still needs to be studied under different point of views and for which an anesthetization protocol has to be fully evaluated. This research has shown that the ketamine–diazepam drug combination is the anesthetic combination with the fastest onset time and shortest recovery time compared to others such as ketamine–acepromazine and ketamine–xylazine combinations. These results provide new and important information for the medical treatment of an animal species, which is yet to be sufficiently studied. Results from these type of studies are increasingly pertinent due to the effect of climate and environmental changes, currently impacting the worldwide turtle populations.

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Conflicts of Interest

There are no conflicts of interest.
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