



## Evaluation of diazepam-ketamine combination for immobilization of African land tortoise (*Testudo graeca*)

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**Publication History:**  
Received: 18-10-2018  
Accepted: 02-01-2019

### Abstract

Zoo and wildlife practitioners are constantly exposed to persistent dangers during physical restraint of wild animals. Chemical immobilization in reptiles is unpredictable due to their ectothermic nature. This study aims to determine a safe and effective anesthetic protocol for immobilization in chelonians and other reptiles. Varied doses of diazepam ketamine combinations were administered and evaluated in 16 healthy land tortoises (*Testudo graeca*). The tortoises were divided into four groups (4 per group) labeled DK-1 through DK-4. DK-1 had a combination of 44mg/kg of ketamine with 0.25mg/kg of diazepam. DK-2 had 22mg/kg of ketamine with 0.25mg/kg of diazepam. DK-3 had 44mg/kg of ketamine with 0.5mg/kg diazepam while DK-4 had 22mg/kg of ketamine with 0.5mg/kg of diazepam. Anesthetic effects were monitored to determine the duration required for partial extension of the head and limbs, full extension of limbs, and complete recovery from anesthesia. These visual inspection of partial recovery and full extension were adapted as surface and deep anaesthesia respectively in describing the depth of anaesthesia. All the time intervals were recorded in minutes, and summarized as mean and standard deviation. ANOVA was used to test for significance across the groups. Full extension of head and limbs was achieved within mean periods of 10, 15.5 and 13 minutes in DK-1, DK-3 and DK-4 respectively. Tortoises in DK-2, which were only sedated, demonstrated only surface depth of anaesthesia. Complete recovery occurred in mean periods of 128, 25, 158 ( $p < 0.05$ ) and 132 minutes for groups DK-1, DK-2, DK-3, and DK-4 respectively. Diazepam-Ketamine anaesthetic cocktail provides a safe protocol for chemical restraint in tortoises. A higher dose of diazepam produced a longer duration of complete recovery.

**Keywords:** Anaesthesia, Diazepam, Immobilization, Ketamine, *Testudo graeca*

### Introduction

Chemical immobilization is a very important and an unavoidable ancillary to physical restraint in wildlife and zoo animal practice (Fowler, 2008). It involves the use of anesthetic drugs to restrain animals for research, medical intervention and sample collection for the laboratory (Brothers, 2010). Generally, five categories of drugs are associated with chemical

immobilization in zoo and wild animals. They include tranquilizers, paralytic drugs, narcotics, sedatives, and dissociative anesthetics. This has made possible medical interventions and manipulative procedures that were hitherto impossible in the past, thus saving the lives of many animals by the judicious use of drugs to minimize stress and trauma. Chemical

anesthesia in reptiles was reported to be difficult due to varied induction, duration and recovery periods as they are usually longer in reptiles than mammals (Jayathangaraj & John, 1999). According to Boyer (1992), immobilization of reptiles can be unpredictable, when compared to other wild animals, due to their ectothermic nature (inability to regulate their body temperature).

Diseases affect chelonians in free and captive states requiring medical or surgical interventions. Appropriate combination and use of chemical agents for immobilization is a requirement for safe restraint and capture of any wild animal species in a free range or in captive state (Nielsen, 1999). Ketamine has been used successfully in all reptilian orders, due to its high safety margin when compared to other anaesthetics agents, as well as the possible diverse routes of administration in both wild and domestic animals species (Dupras *et al.*, 2001; Alves-Junior *et al.*, 2012; Adejumobi & Olukole, 2016; Ogunsola & Adetunji, 2018).

Zoo and wildlife veterinarians, as well as zoo and wildlife workers, are exposed to lots of dangers when wild animals are to be restrained. This may consequently create public safety issues like fatal attacks on humans and other animals and threats to the animal's own wellbeing (Bill, 2010). A safe and effective anaesthetic protocol is therefore, essential for the various medical procedures and interventions both on the field and in the wild, or in zoo hospitals (Ogunsola & Adetunji, 2018). The objective of this study, therefore was to evaluate the anaesthetic effects of concurrent administration of varied doses of ketamine and varied doses of diazepam in apparently healthy tortoises, by determining the depth of anaesthesia and time required for full recovery in tortoises administered with different cocktails of the anaesthetic agents. It is expected that data from this study will provide useful information that will further the surgical health management of chelonians.

## Materials and Methods

### Ethical statement

All necessary permits were obtained from the Animal Care Use and Research Ethics Committee (ACUREC) University of Ibadan, Nigeria (Reference number UI-ACUREC/App/10/2016/001).

### Animals and stabilization

Sixteen (16) land tortoises *Testudo graeca* with mean weight of 0.93kg, sourced from local farmers in Ibadan, Nigeria were used in this study. The animals were housed in a wooden vivarium constructed and demarcated into eight apartments. The tortoises were weighed and housed in twos. They were also prophylactically treated with levamisole hydrochloride 25mg/kg, 20% oxytetracycline and Vitamin B Complex 50mg/kg. The animals were fed *ad libitum* with fruits and pelletized feed.

### Anesthetic drugs and formulations

Diazepam (Laborate® Pharmaceuticals, India) supplied as 10mg/ml solutions for intramuscular and intravenous injection in a 2ml vial, and ketamine hydrochloride (Laborate® Pharmaceuticals, India) supplied as 50mg/ml solutions for intramuscular and intravenous injection in a 10ml multidose vial were used.

### Experimental procedure and anesthetic protocols

Tortoises were marked for identification using prefix of their species and Arabic numerals: TG (*Testudo graeca*) 1-16. They were divided into four groups containing four animals each. The tortoises in the four groups of the Diazepam-Ketamine (DK) protocol received varied doses of the anesthetics as intramuscular injections on the right forelimb. The selected doses were chosen based on results of previous pilot studies by the authors. DK-1 received 0.25mg/kg diazepam and 44mg/kg ketamine. DK-2 received 0.25mg/kg diazepam and 22mg/kg ketamine. DK-3 received 0.5mg/kg diazepam and 44mg/kg ketamine. DK-4 received 0.5mg/kg diazepam and 22mg/kg ketamine. The effects of the anesthetics agents were observed and recorded. The parameters recorded (in minutes) included: (a) time to partial extension of the head and limbs (described as the time interval between ketamine injection and the swift retraction of tortoise's head and limbs when touched with a pair of forceps), also known as surface anaesthesia (b) duration of full extension (described as the time interval between head or limb retraction after ketamine injection and when no movement or retraction of the head, limbs, tails when touched, also known as deep anaesthesia, and (c) complete recovery from anesthesia (described as duration for the tortoises to regain full activities).

**Table 1:** Diazepam-Ketamine combination for chemical immobilization in *Testudo graeca*

	DK-1 (n=4)	DK-2 (n=4)	DK-3 (n=4)	DK-4 (n=4)
Partial Recovery (mins)	9.5 ± 1.29 <sup>b</sup>	23.2 ± 2.36 <sup>a</sup>	10.8 ± 2.22 <sup>b</sup>	10.0 ± 1.29 <sup>b</sup>
Full Extension (mins)	10.0 ± 1.03 <sup>a</sup>	NA	15.5 ± 1.29 <sup>a</sup>	13 ± 2.58 <sup>a</sup>
Complete Recovery (mins)	128 ± 4.23 <sup>b</sup>	25 ± 1.41 <sup>a</sup>	158 ± 6.48 <sup>c</sup>	132 ± 10.58 <sup>b</sup>

Values with different superscripts across groups (DK1 through DK4) are significantly different

Body weight of the sixteen tortoises = 930 ± 186 grammes

DK-1: 0.25mg/kg diazepam and 44mg/kg ketamine; DK-2: 0.25mg/kg diazepam and 22mg/kg ketamine; DK-3: 0.5mg/kg diazepam and 44mg/kg ketamine; DK-4: 0.5mg/kg diazepam and 22mg/kg ketamine; NA: Depth of anaesthesia did not reach this level

#### Data analysis

Data which included body weight (in grammes) of tortoises and time intervals (in minutes) recorded for the various parameters outlined above, were analyzed using SPSS v20. The data were summarized as mean ± standard deviation. Analysis of variance was used to compare means across groups. Statistical significance was recorded as  $p < 0.05$ .

#### Results and Discussion

The mean ± standard deviation of the body weight of the sixteen tortoises was 930 ± 186 grammes. The depth of anaesthesia in tortoises in DK2 was only surface anaesthesia. There was no full extension (deep anaesthesia). Tortoises were only sedated and recovered fully between 23 – 26 minutes. Details of the various times recorded are presented in Table 1. Chelonians are ectotherms and depend on the temperature of their environment to regulate their body temperature. Therefore the preferred optimal temperature zone 26-38°C (POTZ) was considered (Boyer & Boyer, 2006). Arising from their study on the anesthetic effect of ketamine alone, and ketamine combinations in a range of animal species, Green *et al.* (1981) reports that ketamine alone has several limitations in terms of prolonged recovery time but produced a good anaesthesia when used in cocktail.

We found that Group DK-2 only demonstrated surface anaesthesia that did not progress to deep anaesthesia. The reason for this finding is inexplicable from this study. This finding negates the findings of Fowler (2008) that there was no need to exceed 12.5mg/kg ketamine dosage in a bid to achieve anaesthesia. However, it is noteworthy to mention that their findings were observed in turtles. Species differences might account for the different observations in anaesthetic depth.

Although, there was no significant statistical difference in the times recorded for partial recovery (surface anaesthesia) and full extension (deep anaesthesia) for groups DK-1, DK-3 and DK-4, there

was significant statistical difference in the times for complete recovery across the three groups. Groups DK-1, DK-3 and DK-4 produced anaesthesia that required over two hours for tortoises to attain full recovery. This experience with ketamine is in agreement with previous works that ketamine produced prolonged recovery that can take hours to days (Bennett, 1996; Carpenter *et al.*, 1996). Expectedly, DK-3 with the highest dosages of both drugs produced a longer duration of complete recovery. The results of this study suggest that the dosage of diazepam may play a role in determining the time required for full recovery. Groups that received 0.5mg/kg diazepam (DK-3 and DK-4) required relatively longer times for full recovery than Groups that received 0.25mg/kg diazepam (DK-1 and DK-2)

The duration of the medical or surgical intervention would play a role in determining what choice of protocol to be employed. More sensitive markers of depth of anaesthesia and analgesia should be employed in future studies to determine what protocol would work best for specific procedures and interventions. We conclude that diazepam-ketamine cocktail can provide suitable anaesthesia with full recovery in land tortoises. The dose of diazepam rather than that of ketamine determines the duration required for full recovery.

#### Acknowledgement

The authors thank Mssrs. Omotayo Abiodun and Omoh for their assistance.

#### Conflicts of Interest

The authors declare they have no conflict of interest.

#### References

- Adejumobi OA & Olukole SG (2016). Ketamine-induced immobilization in the African sideneck turtle (*Pelusios castaneus*). *European Journal of Pharmaceutical and Medicinal Research*, 5(3):36-37.

- Alves-Junior JRF, Bosso ACS, Andradel MB, Werther K, Luiz A & Santos Q (2012). Association of midazolam with ketamine in giant Amazon River turtles (*Podocnemis expansa*) bred in captivity. *Acta Cirúrgica Brasileira*, **2**(27): 145-147.
- Bennett RA (1996). Anesthesia, In: *Reptile Medicine and Surgery* (DR Mader, editor), WB Saunders Co., Philadelphia. Pp 241-247.
- Boyer TH & Boyer DM (2006). Turtles, Tortoises and Terrapins. In: *Reptile Medicine and Surgery* (DR Mader, editor), Second edition St. Louis: Elsevier Science. Pp 78–99.
- Boyer TH (1992). Clinical anaesthesia for reptiles. *Bulletin of the Association of Reptilian and Amphibian Veterinarians*, **2**(2): 10-13.
- Brothers B (2010). Operational guide for animal care and control agencies: Chemical capture. *American Humane Association*. Pp 1 – 14.
- Carpenter JW, Mashima TY & Rupiper DJ (1996). Reptiles. In: *Exotic animal formulary* (JW Carpenter, TY Mashima, DJ Rupiper editors). Greystone Publications, Manhattan, Kans. Pp 62.
- Dupras J, Vachon P, Cuvelliez S & Blais D (2001). Anaesthesia of the New Zealand rabbit using the combination of tiletamine-zolazepam and ketamine midazolam with or without xylazine. *Canadian Veterinary Journal*, **42**(6): 455-460.
- Fowler ME (2008). Restraint and Handling of Wild and Domestic animals. Third edition. Wiley-Blackwell, Iowa, USA. Pp 415-416.
- Green CJ, Knight J, Precious S & Simpkin S (1981). Ketamine alone and combined with diazepam or xylazine in laboratory animals: A ten year experience. *Laboratory Animal*, **15** (1): 163-170.
- Jayathangaraj MG & John MC (1999). Chemical immobilization and haematological sampling in an Indian flapshell turtle (*Lissemys punctata*). *Zoos' Print Journal*, **14**(7): 70-71.
- Nielsen L (1999). Chemical Immobilization of Wild and Exotic Animals. Iowa State University Press; Ames, Iowa, USA. Pp 277-280.
- Ogunsola J & Adetunji V (2018). Use of diazepam and ketamine anaesthesia in prevention of capture myopathy in the ostrich (*Struthio camelus*). *Sokoto Journal of Veterinary Sciences*, **16**(1): 86-89.