

EFFECTS OF MIDAZOLAM IN DIFFERENT DOSES IN REDTAIL BOA *Boa constrictor* LINNAEUS, 1758 (SQUAMATA: BOIDAE)

EFEITOS DA ADMINISTRAÇÃO DO MIDAZOLAM EM DOSES DIFERENTES EM JIBOIAS *Boa constrictor* LINNAEUS, 1758 (SQUAMATA: BOIDAE)

Simone Borges Salgueiro De Simone¹

Líria Queiroz Luz Hirano^{1*}

André Luiz Quagliatto Santos¹

¹Universidade Federal de Uberlândia, Uberlândia, MG, Brazil.

*Author for correspondence - liriaqueiroz@yahoo.com.br

Abstract

The objective of this study was to compare the effects of midazolam in *Boa constrictor*. We used 20 redtail boas, divided into two groups of ten animals each. Group 1 (G1) received 1 mg/kg of midazolam and group 2 (G2) 2 mg/kg, by intracelomic injection. The specimens of G1 presented reduction of head tone, muscle tone, manipulation, and locomotion for 233.50 ± 71.34 minutes and the representatives of G2 for 328.50 ± 125.35 minutes. No redtail boa belonging to both G1 and G2 presented absence of postural righting reflex or achieved analgesia. However, all specimens tested showed intense muscle relaxation and difficulty to move in. The turnaround time to pre-sedation conditions were 279 ± 73.55 minutes for G1 and 372 ± 142.27 minutes for G2. There were no statistical differences between evaluations of heart and respiratory rates in both groups tested. We concluded, therefore, in redtail boa, midazolam at the dosage of 1 mg/kg causes the same effect as 2 mg/kg, but with shorter recovery time, and it can be used to contain *Boa constrictor* or in associations aimed at an effective muscle relaxation.

Keywords: benzodiazepinic; pharmacological restraint; reptiles; snakes.

Resumo

Objetivou-se comparar os efeitos de duas doses de midazolam em *Boa constrictor*. Utilizaram-se 20 jiboias, divididas em dois grupos. O grupo 1 (G1), composto por dez animais, recebeu 1 mg/kg de midazolam e o grupo 2 (G2), também com dez animais, 2 mg/kg, pela via intracelomática. Os animais do G1 apresentaram déficit de tônus da cabeça, tônus muscular, manipulação e locomoção por $3,89 \pm 1,18$ horas e os representantes do G2 por $5,47 \pm 2,08$ horas. Nenhuma jiboia, em ambos os grupos, manifestou ausência de reação postural de endireitamento ou alcançaram sedação profunda; entretanto, observou-se redução da agressividade, intenso relaxamento muscular e uma dificuldade de serpentear em todos os exemplares. O tempo de retorno às condições pré-sedativas foi de $4,65 \pm 1,22$ horas para o G1 e de $6,2 \pm 2,37$ horas para o G2. Não foram observadas diferenças significativas nas avaliações da frequência cardíaca e respiratória entre os grupos. Concluiu-se assim que 1 mg/kg de midazolam provoca nas jiboias o mesmo efeito que 2 mg/kg, porém na menor dose o tempo de recuperação é menor, podendo ser utilizado para a contenção farmacológica de *Boa constrictor*, ou em associações que visem um efetivo relaxamento muscular.

Palavras-chave: benzodiazepínico; contenção farmacológica; répteis; serpentes.

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Introduction

The main objective of breeding boas in captivity is to supply the exotic and wild animals' market of snakes⁽¹⁾. Properly performed chemical restraint is fundamental for the success of wild animals clinic. Stress is a set of reactions of the organism facing physical, psych, infectious and other types of aggressions that cause homeostasis break, and it is a major factor that should be considered. Capture, transportation, material collections for examinations, and treatments are relevant stress-causing factors, and the prolonged stimulus of the physical restraint may lead the animals to its death⁽²⁾. Therefore, the objective of the chemical restraint is to minimize the risks for both the animal and the handler⁽³⁾.

Although there are reports on the lack of necessity of pre-anesthetic drugs (PAD) in reptiles⁽⁴⁾, other authors have suggested the use of α -2 agonists, benzodiazepines, or anticholinergics for a better muscle relaxation, reduction of the necessary dose of anesthetics, and to perform proper restraint^{(5),(6),(7)}. Among the advantages of these drugs stand out the loss of medullary reflexes and muscle activity, besides the reduction of aggressiveness, a fundamental point regarding wild animals⁽⁸⁾.

Benzodiazepines are tranquilizing drugs that act on the central nervous system (CNS), potentialize the effects of the gamma-Aminobutyric acid (GABA), due to the increase in the connection affinity between GABA receptor and its primary transmitter. Although they are broadly employed as pre-anesthetic drug or in the chemical restraint of mammals, there are a only few studies on the isolated use of benzodiazepines in reptiles^{(8),(9)}. In snakes, the employment of these drugs is limited to the report of the use of midazolam in little invasive procedures or during blood collections or diagnostic imaging, without details of the sedative effects or the duration of the stages⁽¹⁰⁾.

Midazolam, a 1.4-benzodiazepine-derived benzodiazepine, can promote adequate miorelaxation, reducing muscle tonus; however, it may lead to hypotension and bradycardia in mammals⁽⁸⁾. In crocodylians of the species *Crocodylus porosus*, the use of this drug is advantageous in chemical restraint for the collection and transportation of biological samples, with the decrease in the biochemical levels of stress markers when compared with the physical restraint, and the reduction of the heart rate is insignificant⁽⁹⁾.

Due to the scarcity of protocols for the chemical restraint of snakes and the lack of studies with the isolated use of benzodiazepines in these animals, we aimed at assessing and comparing the clinical, cardiovascular, and respiratory effects of the midazolam at the doses of 1 and 2 mg/Kg, intracoelomic via (ICe), in *Boa constrictor*.

Material and Methods

We carried out the experiment at the laboratory of teaching and research of wild animals (LAPAS) of Universidade Federal de Uberlândia - UFU, with 20 *Boa constrictor* specimens. The animals were healthy and of both sexes. The Instituto Chico Mendes of Biodiversity Conservation (ICMBio) authorized the experiment under the license No. 24326-1 as well as the Ethics Committee in the Use of Animals of Universidade Federal de Uberlândia (CEUA), under the No. 092/10.

The animals were weighed and submitted to physical examination 15 days before the chemical

restraint to certify the health condition of the snakes. In the evaluation of the general health state, we observed the degree of hydration by the elasticity of the skin and ocular brightness pattern, body score by the visualization and palpation of the paravertebral musculature, and alteration in the color of mucosa and presence of secretion by the examination of the oral cavity.

Furthermore, we evaluated the cardiac function by assessing heart frequency and rhythm (HR) with a vascular doppler device, and the respiratory rate (RR) by the observation of respiratory movements and auscultation with the use of a stethoscope. The behavioral patterns of aggressiveness or fleeing in the face of human presence, interest and capture of live prey were also observed. Copro-parasitological examination was performed⁽⁶⁾. We also verified that no animal was undergoing ecdysis process during the experiment execution, which could interfere with the animal's metabolism⁽¹¹⁾.

The specimens were placed individually in plastic boxes, which were numbered to identify the animals. The animals were submitted to 15-day food fast. They were transferred to the experimental anesthesia room 12 hours before the anesthetic procedure, where they remained in water fast. The room temperature was monitored by a digital thermo-hygrometer hourly. The temperature was kept within the thermal comfort range for boas (25 to 30 °C) by the use of 100W light bulbs to avoid thermic interferences from the environment in the reptiles metabolism while waiting for the drugs to act^{(12),(13)}.

A hook was used for the physical restraint, providing more security to the executing team. From the identification number of the animals, they were distributed randomly by a randomizing software (Random.org, Randomness and Integrity Services Ltd., Dublin, Irlanda) into two groups made of five females and five males each. The five first animals from both sexes listed by the software constituted group 1 (G1). The mean weight of the females was 2.11 ± 1.10 kg and of the males was 0.92 ± 0.50 kg. The animals received 1 mg/kg of midazolam. Group 2 (G2) was composed of females and males with the mean weight of 1.58 ± 0.28 kg and 0.94 ± 0.34 kg, respectively, and they received 2 mg/kg of midazolam.

In both protocols, the tranquilizing agent was applied in the coelomic cavity, after antisepsis with gauze soaked in 70° GL alcohol, with the aid of 1 mL sterile syringes, coupled to 13 x 0.45 mm hypodermic needles, at the final third of the body, more precisely on the median ventral line between the scales (Figure 1B)⁽¹⁴⁾.

Before the drug application, which corresponded to the basal moment (BM), we evaluated the physiological parameters of heart rate for a minute using a vascular doppler device (Figure 1A) and respiratory rate (RR) by the visualization of the respiratory movements. Moreover, we evaluated the nociceptive and motor parameters presented below⁽¹⁵⁾:

- Sensitive blockage: classified as absent or present by the observation of muscle contractions and attempt of fleeing or aggression by the stimulation of a 13 x 4.5 mm hypodermic needle on the skin and paravertebral musculature at the dorsal region, at the end of each third of the animal's body. This methodology is used in studies on dogs anesthesia^{(16),(17)}, but there are no reports on its use in snakes. Therefore, it was established by pilot studies that showed that clamping the skin of these animals was hindered by the little elasticity and the high adhesion to the musculature. The sensitivity evaluation by hypodermic needle proved to be effective for monitoring the nociception, producing the response of distancing from the respectively stimulated body region.

- Motor blockage: determined by the observation of the head tonus (characterized by the capacity of the specimen to sustain the head); muscular tonus (evaluated by the absence of muscular contraction in the entire body of the animal); locomotion pattern (where the easiness and speed of locomotion was observed by means of manual stimulation capable of promoting snaking movements); easiness of manipulation (characterized by the acceptance of opening the mouth by the handler). These parameters were classified as (1) when in correspondence with BM responses, (2) when they presented intermediate response, and (3) when the response was absent. The observation of these signs indicated the presence of muscular relaxation.

- Straightening postural reaction (SPR): the animals were positioned in dorsal decubitus and the

capacity and time to return to the anatomical position of the species were observed. The return within 1 minute was considered present, and after this period, absent.

Furthermore, three periods of midazolam effects were determined from the moment of application until the complete recovery of the animals (Table 1)⁽⁵⁾.

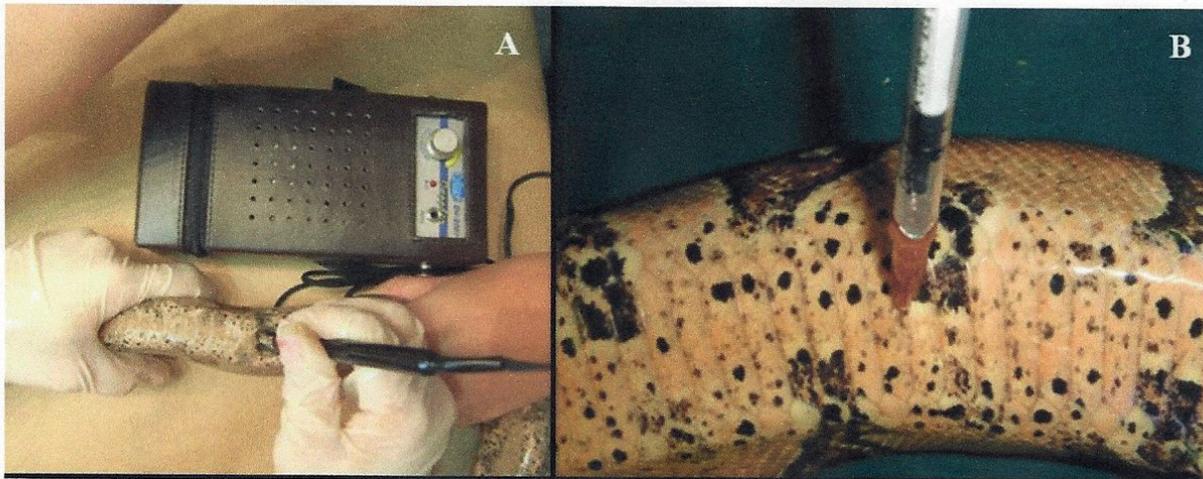


Figure 1. A- Monitoring of the heart rate of *Boa constrictor* with the aid of a vascular Doppler device. B- Midazolam application (intracoelomic via) in *Boa constrictor*.

Table 1. Periods of pharmacological restraint promoted by midazolam in *Boa constrictor*

Period	Beginning	End
Latency	Drug application	Beginning of the effect of the drug (loss of muscular tonus, head tonus, locomotion, pain sensitivity, and aggressiveness)
Able	Loss of the straightening postural reaction	Return to the straightening postural reaction
Recovery	Return to the straightening postural reaction	Return to the pre-anesthetic state of the muscular tonus, head tonus, locomotion, pain and easiness of manipulation

All the parameters previously mentioned were evaluated at the BM and after the application of midazolam, so that in the first 60 minutes, the monitoring took place every 15 minutes, and after the first hour, in 30-minute intervals until the complete recovery of the animals.

With the objective of verifying the existence of significant differences for the intervals of the chemical restraint phases between both groups, as well as the difference between HR and RR at the BM and the other moments, we applied Mann-Whitney U-test and compared the series of data, two by two, with a significance level of 5% in a bilateral test.

Results and Discussion

The latency phases of the animals in G1 and G2 did not differ significantly. Fifteen minutes after the application of midazolam, we observed a discrete muscle relaxation (score 2) in 85% of the animals. The other animals reached score at 30 minutes after the drug administration. The findings of the current study contradict Brazenor and Kaye⁽¹⁸⁾ and Bonath and Zschege⁽¹⁹⁾, who reported a midazolam induction period in reptiles varying from 40 to 60 minutes. However, the authors did not mention the room temperature control, and the drug application in these studies was intramuscular, which may influence the metabolism and the absorption of the benzodiazepine.

In mammals, the benzodiazepine reduces the aggressiveness, cause amnesia and psychomotor alterations, have anxiolytic, myorelaxant action, and almost no analgesic action⁽⁸⁾. In reptiles, reduction in aggressiveness, muscle relaxation, and psychomotor alterations have been reported. In crocodylians, the IM application of 5 mg/kg of midazolam reduced the level of biochemical stress markers significantly and had a positive result on post-sedation behavior when compared with the isolated physical restraint. Some authors suggest this can be a sign that in these animals benzodiazepines also cause amnesia⁽⁹⁾. In the evaluated boas, we did not observe an alteration in the behavior after sedation and all the animals ate up to seven days after the chemical restraint.

In both protocols, the maximum level of sedation accomplished was the stage 2 defined by Malley⁽¹⁵⁾. This finding was confirmed by the presence of intense muscular relaxation, inability to sustain the head, and reduction or absence of aggressiveness. The animals in G1 remained in this stage for 233.50 ± 71.34 minutes and animals in G2 for 328.50 ± 125.35 minutes (Table 2). The IM application of 1.5 mg/kg in *Trachemys scripta*, also kept in optimum temperature for reptiles, caused a mean restraint time of 82 minutes, inferior to the time in the present studies⁽²⁰⁾. These variations in the individual responses and between species remark the importance of studies on drugs for the chemical restraint of reptiles from various subclasses.

Even when the animals reached effective muscular relaxation, characterized by locomotion difficulty by the snakes, which was verified by the presence of spasms of incoordination, we did not observe loss of straightening postural reaction (SPR). Also, the specimens did not reach the regular time of anesthesia, which according to Bennett⁽⁵⁾, is defined as the interval between the loss and the return of straightening postural reaction (Figure 2).

Table 2. Means and standard deviation (σ) about the time the specimens of *Boa constrictor* remained in stage 2 of muscle tonus (MT), head tonus (HT), manipulation (Manip), and locomotion (Loc.) with the use of 1 mg/kg or 2 mg/kg of midazolam, intracoelomic via

Variables	Midazolam 1 mg/kg		Midazolam 2 mg/kg	
	Stage 2*		Stage 2*	
	Mean	σ	Mean	σ .
MT	233.5a	71.34a	328.50b	125.35b
HT	233.5a	71.34a	328.50b	125.35b
Manip	233.5a	71.34a	328.50b	125.35b
Loc	233.5a	71.34a	328.50b	125.35b

* Values followed by similar letters did not differ statistically (Mann-Whitney U-test at 5%).

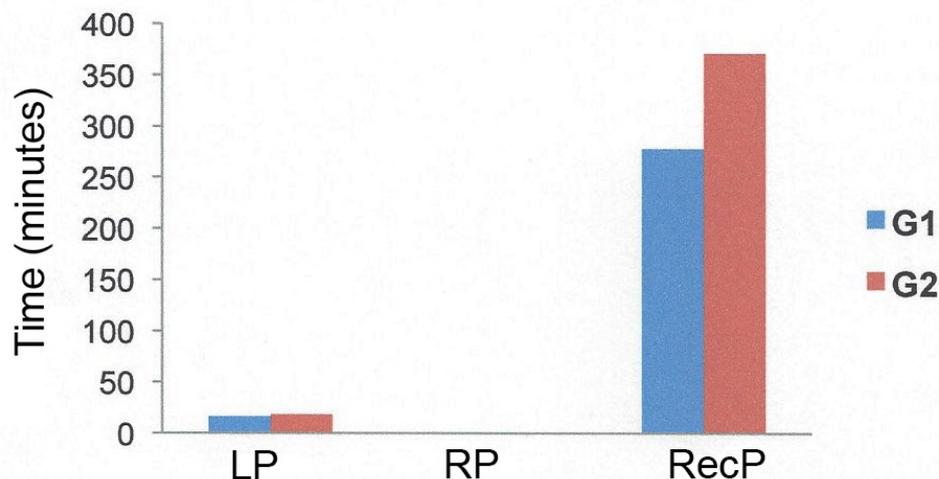


Figure 2. Representation of the latency period (LP), regular period (RP), and recovery period (RecP) in minutes, of *Boa constrictor*, restrained chemically by midazolam at 1 mg/kg (G1) and midazolam 2 mg/kg (G2), intracoelomic via.

Other studies with midazolam at doses of 1 and 2 mg/kg, IM, in reptiles of the Testudinata subclass also revealed the easiness of manipulation and muscular relaxation of the individuals with mild sedation^{(20),(21)}. The snakes of the current study presented reduction of aggressiveness; however, they did not reach deep sedation. For this reason, the association of this benzodiazepine with other drugs, such as dissociative and opioids, is necessary for a more intense effect in invasive procedures⁽²²⁾.

The proposed protocols are not indicated in isolation for surgeries because they do not promote absence of response to the nociceptive stimulus. Nevertheless, the animals presented lethargic behavior, they were indifferent to the environment and humans, and when stimulated by the hypodermic needle, they showed small fleeing reactions to the stimulus.

The recovery time of the animals from G1 was 279.00 ± 73.55 minutes, while in G2 this fact occurred at 372.00 ± 142.27 minutes, values with significant difference ($p < 0.05$) (Figure 3). There are no studies showing the isolated use of benzodiazepines in snakes and the intervals of this type of protocol described for Testudinata specimens vary among species and according to the association used^{(20),(21)}. Bienzie and Boyd⁽²¹⁾ compared the application of 2 mg/kg of isolated midazolam with protocols containing isolated ketamine (40 mg/kg) or associated to the benzodiazepine (2 mg/kg of midazolam associated to 20 mg/kg of ketamine) in *Chelydra serpentina*. The authors reported that at 210 minutes, all the specimens had recovered and the association of the drugs potentialized the effects of sedation, without prolonging the return significantly to basal conditions. This observation reinforces the recommendations of a 2 mg/kg midazolam dose for *Boa constrictor* along with the need of performing new studies comparing the association of sedative drugs and its effects in snakes.

As they did not present differences between the intensity of the effects and the recovery time of 279 minutes is considerably long, the dose of 1 mg/kg, intracoelomic via, is indicated for snakes of the *Boa constrictor* species. According to Spinosa⁽⁸⁾, an increment in benzodiazepine doses does not increase the tranquilizing degree, only the adverse effects. This fact could not be verified in the current study, because the double of the dose just prolonged the period of sedation, without incrementing the desirable effects of the use of benzodiazepines (Table 3).

Similarly to the study of the isolated use of midazolam in *Trachemys scripta*⁽²⁰⁾, significant differences in the heart rate between G1 and G2 or within the groups regarding the BM in boas were not observed. The HR varied from 27 to 77 bpm (Figure 4).

Bennett⁽¹²⁾ stated that reptiles might remain in apnea for up to 24 hours, performing percutaneous and anaerobic respiration. In the current study, we chose to evaluate the RR and not the apnea time as Carregaro et al. ⁽²³⁾ studying rattlesnakes, once boas did not present prolonged apnea after the benzodiazepine application. The presence of apnea in rattlesnakes was caused by the use of ketamine, which is a drug with dissociative action. Regarding the midazolam, the absence of apnea was expected because there are no reports of this event when the benzodiazepine is used isolatedly in reptiles ^{(9),(18)}. We did not observe differences in the RR between the animals from G1 and G2 as well as in the studies of the isolated use of midazolam in *Trachemys scripta* and *Crocodylus porosus*, where the authors reported the absence of bradypnea^{(9),(20)} (Figure 5).

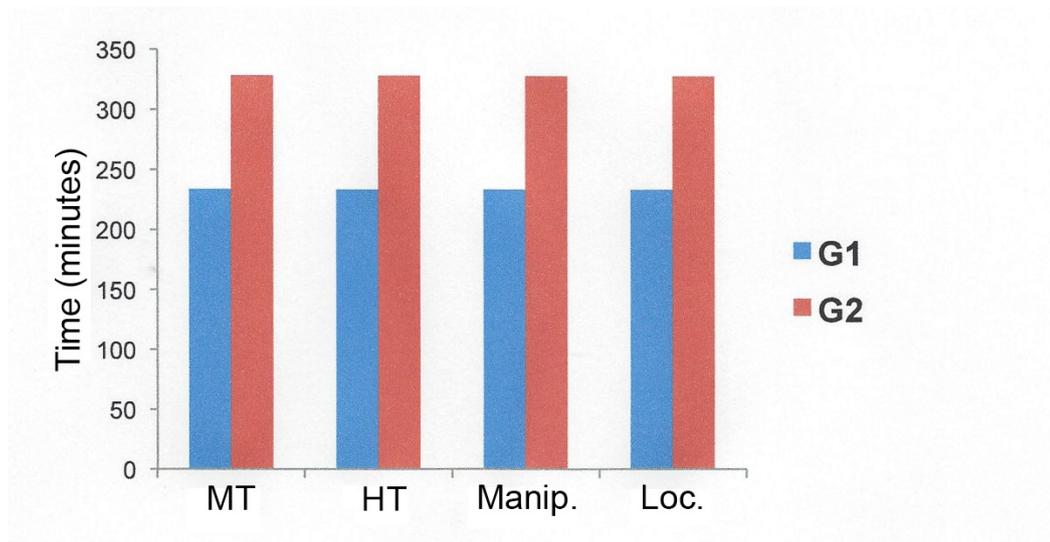


Figure 3. Representation of the mean values of the anesthetic duration regarding muscular tonus (MT), head onus (HT), manipulation (Manip), and locomotion (Loc) in *Boa constrictor* with the use of midazolam at 1 mg/kg (G1) and midazolam at 2 mg/kg (G2), intracoelomic via (ICe).

Table 3. Effects obtained with midazolam at 1 mg/Kg (G1) and midazolam at 2 mg/kg, intracoelomic via, at the evaluation of tranquilization, muscular relaxation, restraint, analgesia, heart rate (HR), respiratory rate (RR), return to the conditions of the basal moment (BM), and little or non-invasive procedures in *Boa constrictor* specimens

Analyzed Variables	Midazolam 1 mg/kg	Midazolam 2 mg/kg
Tranquilization	yes	yes
Muscular relaxation	yes	yes
Restraint	yes	yes
Analgesia	No	No
HR	No alteration	No alteration
RR	No alteration	No alteration
Return to BM	279 ± 73.55	372 ± 142.27
Little or non-invasive procedures	No	No

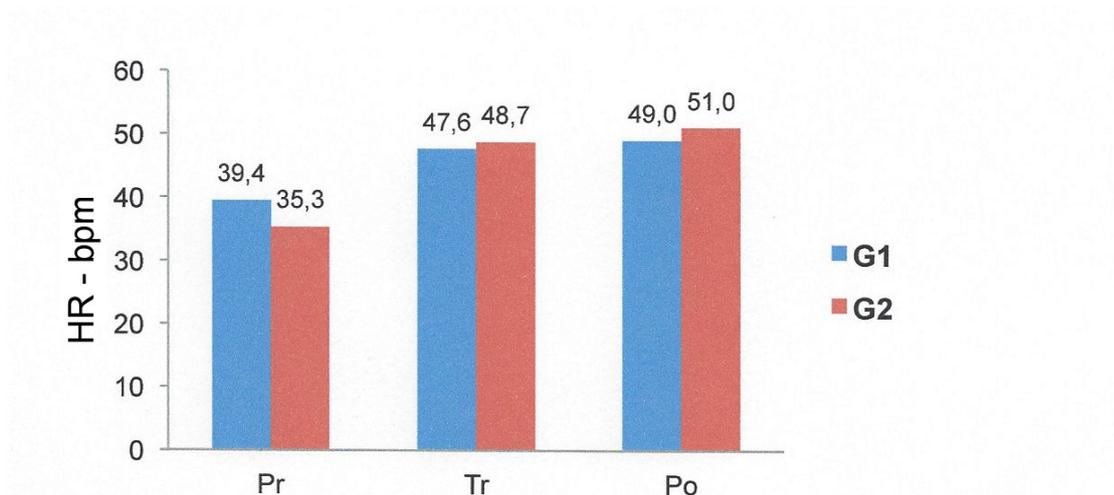


Figure 4. Representation of the mean values of the heart rate (HR) in beats per minute (BPM) at the periods pre-sedation (Pr), trans-sedation (Tr), and post-sedation (Po) in boas with the use of midazolam at 1 mg/kg (G1) and midazolam at 2 mg/kg (G2), intracoelomic via (ICe).

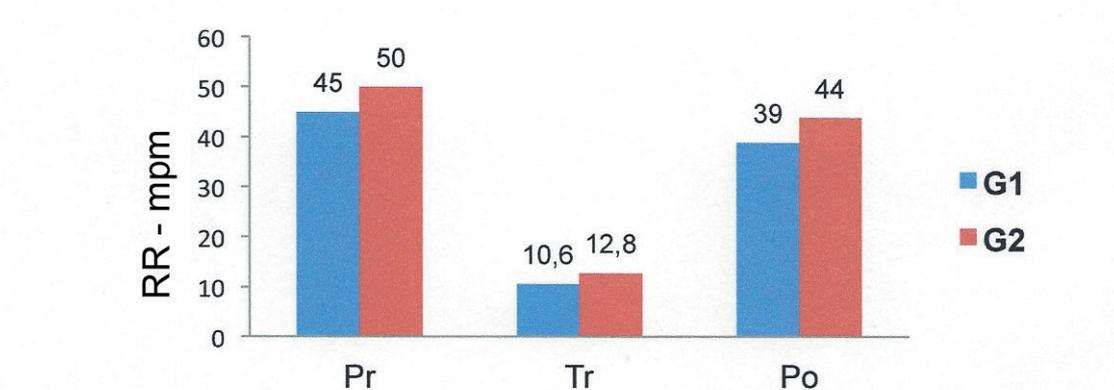


Figure 5. Representation of the mean values of the respiratory rate (RR) in movements per minute (mpm) in the periods pre- (Pr), trans- (Tr) and post-anesthetic (Po) in boas with the use of midazolam 1 mg/kg (G1) and midazolam 2 mg/kg (G2), intracoelomic via (ICe).

According to West et al.⁽²⁴⁾, snakes with moderate to severe dehydration levels must receive fluids reposition ICe so that there is an improvement in the absorption of the administered substances. Searching a better anesthetic action, we also chose the intracoelomic via; however, the comparison of the intensity and the latency period of other studies on the intramuscular application of midazolam in reptiles did not show a difference between both vias. This result indicates the need pharmacokinetic investigations of drugs administered in the coelomic cavity to better the understanding of the absorption and metabolism of this via.

Conclusion

We verified that midazolam at the doses of 1 and 2 mg/kg in boas promotes safe tranquilization, with effective muscular relaxation and without physiological alterations of the HR and RR; however, it does not promote analgesia, and its isolated use is contraindicated in the performance of surgical procedures. Nevertheless, due to the rapid beginning of the action and the considerable duration of action and recovery, the agent may be used for pharmacological restraint of *Boa constrictor* in little invasive procedures, such as biological samples collection, diagnostic imaging exams, and transportation, or in associations that aim at an effective muscular relaxation. Due to the difference in the recovery time and the absence of advantages regarding the intensity of effects observed in both groups, we indicate the use of 1 mg/kg of midazolam, intracoelomic via, for the pharmacological restraint of *Boa constrictor*.

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