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Short communication

CHEMICAL RESTRAINT IN SOUTHERN TIGER CATS *(LEOPARDUS GUTTULUS)* WITH METHADONE OR MORPHINE ASSOCIATED WITH XYLAZINE-KETAMINE

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The Southern Tiger Cat (*Leopardus guttulus*) is an endangered species, requiring effective anesthesia for clinical procedures, yet species-specific studies are lacking. This study aims to assess and compare the effectiveness of methadone or morphine combined with xylazine-ketamine for the immobilization of Southern Tiger Cats (*Leopardus guttulus*). The animals were randomly allocated to receive sedation with methadone (0.3 mg kg) or with morphine (0.5 mg kg) in association with xylazine-ketamine (1.0 mg kg and 10.0 mg kg, respectively) intramuscularly. Electrocardiography parameters and physiological variables were recorded every five minutes since the application. After 40 minutes of assessment, reversal protocol for opioids and α2-adrenergic agonist was applied using naloxone and yohimbine, and the quality of recuperation was evaluated. In this study, no significant differences were noted between groups or assessment times in the physiological variables monitored. One subject, presented idioventricular accelerated rhythm, attributed to morphine administration and confirmed by its reversal. Recovery evaluation demonstrated favorable outcomes, although some animals presented adverse effects, exacerbated by supplementary ketamine doses. The morphine-xylazine-ketamine and methadone-xylazine-ketamine protocols demonstrated similar efficacy in Southern Tiger Cats (*Leopardus guttulus*), exhibiting stable physiological parameters and

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smooth recovery. The use of antagonists facilitated uneventful recovery, contributing valuable insights into anesthetic protocols for endangered species. The applied protocols provided safe examination and valuable insights into anesthetic protocols for this endangered species.

Keywords: Wild felids, sedation, immobilization, reversal, arrhythmia.

INTRODUCTION

The Southern Tiger Cat (*Leopardus guttulus*) can be found from Costa Rica to Argentina and throughout Brazil, except in the southern grasslands and mangrove formations [1]. The species is listed as 'vulnerable' by the International Union for the Conservation of Nature (IUCN) [2].

Wildcats must be anesthetized to carry out clinical examinations since physical restraint allows a limited number of procedures to be carried out and promotes animal stress and risks of injury to the work team. Therefore, several protocols are described for wildcats' anesthesia [3]. The drug doses are calculated based on references from domestic feline species references. This can result in drug under or overdosing due to physiological differences between species and the impossibility of weighing wild animals before administering the anesthetics. Therefore, the use of drugs with antagonists has been recommended [4]. Apart from facilitating the correction of overdoses, these agents enhance the recovery quality in free ranging animals.

Dissociative anesthetics, especially ketamine, are commonly used to immobilize wild felids [5]. Despite its widespread use, ketamine may induce adverse effects, such as muscle rigidity and seizures, and does not possess a pharmacological antagonist. The combination of α2-adrenergic agonists, such as xylazine, with ketamine is employed to mitigate adverse effects and ensure safer sedation by enabling the possibility of reversal with yohimbine, which reduces their pharmacological effects and recovery time [6].

Additionally, opioids such as methadone and morphine, are commonly combined with ketamine to provide sedation and analgesia. This combination helps reduce the dosage of other medications promotes faster anesthetic recovery, and minimizes potential complications. Naloxone is an opioid receptor antagonist that reverses physiological and behavioral alterations caused by opioid administration [5].

Despite the wide geographical distribution of *L. guttulus*, studies regarding anesthetic procedures on this species are scarce. The objective of this study was to assess the quality of pharmacological restraint, cardiovascular effects and recovery quality from anesthesia following the administration of ketamine and xylazine combined with methadone or morphine and subsequent reversal with the combination of naloxone and yohimbine in Southern Tiger Cats (*Leopardus guttulus*).

MATERIALS AND METHODS

Ethical animal research

This study followed all the relevant national and institutional policies for the care and use of animals. The research was carried out only after its approval by the Ethics Committee for the Use of Animals of the Federal University of the Southern Border (protocol number 23205.001596/2014-21).

Experimental design, area of study and animals

The animals originated from the Bela Vista Biological Refuge, in Foz do Iguaçu – Paraná, Brazil. A total of 11 male and female wild cats (*Leopardus guttulus*) with a body mass of 2.55 ± 0.62 kg were used. After fasting for 24 hours, the animals were captured with nets inside their enclosures, transferred to containment boxes and transported to the Veterinary Hospital. Then they were randomly assigned to two groups. The MeKX Group (n = 5) received a combination of methadone (0.3 mg kg), ketamine (10.0 mg kg) and xylazine (1.0 mg kg) and the MoKX Group (n = 6) received a combination of morphine (0.5 mg kg), ketamine (10.0 mg kg) and xylazine (1.0 mg kg), both administered intramuscularly.

Evaluation of parameters

The animals underwent a general clinical examination, including an ophthalmic evaluation, electrocardiography, video otoscopy, interdigital assessment and blood sampling for laboratory tests. Venous access was performed in the cephalic vein at 10 minutes post drug administration (T10), which allowed fluid therapy maintenance with 0.9% NaCl (5 mL kg hour), using a pump infusion. A digital electrocardiography was used to record the ECG data. The exam was performed with the animal in lateral recumbency, using monopolar and bipolar leads, with a sensibility of 1 cm and 1mv, and a speed of 25 mm sec over 20 minutes. Reference values adopted were proposed by Tilley and Smith for domestic cats [7].

Physiological parameters were monitored at 5-minute intervals from T10 onwards until the animals exhibited signs of recovery. Heart rate (HR) and oxygen saturation in hemoglobin (SpO2) were evaluated using a pulse oximeter placed on the lingual side. Additionally, systolic blood pressure (SBP) was measured using a Doppler while the respiratory rate (*f*R) was observing by monitoring thoracic movements. The quality of the pharmacological restraint was also assessed by observing the presence or absence of the interdigital reflex.

Pharmacological reversal

After 40 minutes (T40) of pharmacological restraint, all the animals received the combination of naloxone (0.04 mg kg) and yohimbine (0.2 mg kg) via the cephalic

vein and were placed back in the restraint box. To determine the anesthesia recovery quality, the Wenger Scale (2010) was used. The animals were monitored until they had completely recovered and were then transferred to their enclosures.

Statistical analysis

Statistical analysis was carried out using the GraphPad Prism program. Initially, the Kolmogorov-Smirnov normality test was performed. Parametric data was submitted to the ANOVA test and paired T-test and non-parametric data to the Kruskal-Wallis and Mann-Whitney tests. The confidence level adopted for significance was 5% ($p<0.05$).

RESULTS AND DISCUSSION

There were no differences between the assessment times or between the groups after administration of the drugs. HR, SBP, SpO2 and *f*R remained stable in both groups throughout the anesthetic procedure (Table 1). There was no difference in the assessment of the quality of pharmacological restraint, either between groups or between assessment times.

Table 1. Physiological Effects of ketamine-xylazine with methadone or morphine in *Leopardus guttulus*

Variable	Group	Time points (minutes)						
		T10	**T15**	**T20**	**T25**	**T30**	**T35**	**T40**
HR	MeKX	144±18	144±12	141±15	125±5	128[120;132]	132±16	129±9
	MoKX	170±26	160±35	138±30	142±28	137±35	138±31	144±31
fR	MeKX	32[28;48]	32[28;48]	32[32;48]	32[32;48]	32[28;48]	32[28;32]	32[28;32]
	MoKX	32[32;48]	32[24;48]	32[20;40]	32[24;48]	32[24;40]	28[24;32]	26[20;32]
SBP (mmHg)	MeKX	146±11	146±11	146±14	149±11	150±16	143±16	144±15
	MoKX	130±20	138±17	134±14	133±12	132±9	121±23	118±17
SpO2 (%)	MeKX	93±2	93±2	93±3	92±2	93±2	93±2	91[91;95]
	MoKX	90±5	90±4	94±3	94±3	93±3	93±3	94±3

Values are presented as mean ± standard deviation for parametric data, or median [min; max] for non-parametric data. Heart rate (**HR**), respiratory rate (**fR**), systolic blood pressure (**SBP**), oxygen saturation hemoglobin (**SpO2**)

All the animals remained without interdigital reflexes and with adequate muscle relaxation for the clinical assessments. Movements of the head, tail and pelvic limbs were observed in five animals, three of the MeKX group and two of the MoKX group and an additional dose of ketamine (5mg/kg, IV) had to be administered 29.4±3.36 minutes after the first dose, for the safety of the work team.

These results reflect the variation in anesthetic response between groups and suggest a shorter duration of sedative effects, particularly in the MeKX group. Previous studies support the use of lower ketamine doses combined with α2 agonists for effective sedation in small felids. For example, a study conducted in the same species successfully used 5 mg/kg of ketamine combined with an α2 agonist for short, non-invasive procedures, demonstrating similar efficacy [8]. Conversely, Iglesias et al. (2020) used the same dose of ketamine as in the present study (10 mg/kg), but in combination with dexmedetomidine, also achieving adequate sedation for similar procedures [9].

The difference in drug combinations may explain the need for additional doses in some animals in the present study. While the combination of ketamine and morphine (MoKX) resulted in a longer sedative effect compared to ketamine and methadone (MeKX), it is important to highlight that opioids such as morphine and methadone induce sedation by acting on opioid receptors in the central nervous system. This action reduces pain perception and decreases neuronal activity, promoting relaxation and sedation. Furthermore, opioids can enhance the sedative effects of ketamine through synergistic mechanisms, as both modulate neural pathways associated with nociception and consciousness. However, this synergy may not be as pronounced as that observed with α2 agonists, which exert more direct and potent sedative effects on regulatory centers of the central nervous system.

Considering higher doses of ketamine and xylazine, such as 15 mg/kg and 2 mg/kg, could prolong the anesthetic effect but may also increase the risk of adverse effects, such as respiratory or cardiovascular depression. Therefore, the chosen initial doses reflect a balance between expected efficacy and safety, based on the available literature. Nonetheless, future studies should investigate the impact of higher doses of these combinations to determine whether they offer advantages in terms of anesthetic efficacy and duration without compromising animal safety.

Regarding electrocardiography outcomes, in group MoKX one subject presented an arrhythmia. Initially, the ECG revealed an HR of 166 beats per minute (bpm), the absence of P waves, wide QRS complexes, lasting 67 milliseconds (msec), and bizarre morphology in all waves. Additionally, the QT interval had a duration of 213 msec. These findings are suggestive of accelerated idioventricular rhythm, originating in the left ventricle

Naloxone was administered intravenously to revert the arrhythmia. Six minutes post-administration, sinus rhythm was observed with an HR of 107 bpm. The P wave amplitude ranged from 0.05 to 0.13 mV, indicating a wandering pacemaker. The PR interval was 73 msec, the QRS complex duration of 53 msec and the QT interval was

reduced to 193 milliseconds. Yohimbine was also administered slowly and continuously, to reverse the effects of the alpha 2-adrenergic drugs. No adverse effects such as nausea, vomiting, or reinstatement of the anesthetic state were observed.

There was no disparity observed in the assessment of anesthesia recovery, as most of the animals exhibited grade 2 (good) recovery. Only one animal exhibited grade 4 recovery (unsatisfactory), and one animal demonstrated grade 1 recovery (excellent), both within the MoKX group (Figure 1). However, animals administered an additional dose of ketamine experienced exacerbated adverse effects during recovery, such as muscle tremors and ataxia.

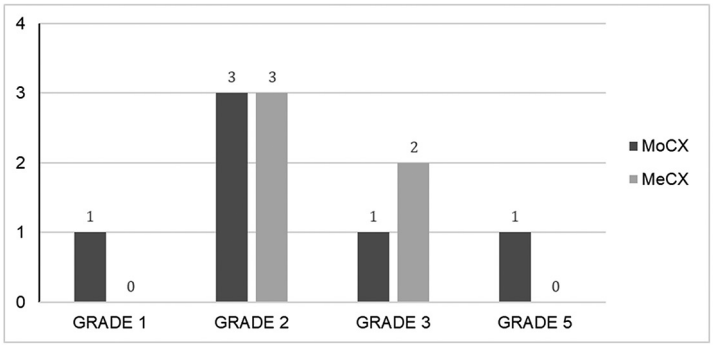

Figure 1. Quality of anesthetic recovery scores for Southern Tiger Cats (*Leopardus guttulus*) following administration of ketamine and xylazine combined with methadone (MeCX) or morphine (MoCX) and subsequent reversal with naloxone and yohimbine. Scores are classified as grade 1 **(excellent)**, grade 2 **(good)**, grade 3 **(regular)**, and grade 4 **(unsatisfactory)**.

In the present study, both groups exhibited maintenance in HR. Although opioids and α2-adrenergic agonists often result in bradycardia, no such effect was observed in this investigation. Opioids exert depressive effects on the cardiovascular system, potentially inducing bradycardia. Similarly, α2-adrenergic agonists can promote bradycardia through their action on presynaptic receptors in the central nervous system. Despite the potential synergistic interaction between these drugs leading to a decline in HR, this effect was not observed in this study, suggesting the safety of the association on Southern Tiger Cats (*Leopardus guttulus*).

The SBP remained stable in both the MeKX and MoKZ groups. Although the drug combination used can cause hypotension, in this study SBP values remained stable at the time of evaluation and within the physiological parameters for domestic cats. Thus, maintaining acceptable SBP values inhibits impaired blood flow and inadequate tissue oxygenation with deleterious changes such as acidosis [1].

Despite the absence of supplemental oxygen, respiratory depression was not observed in any of the groups studied, as evidenced by the *f*R and SpO2 values remaining within the reference values for the species [10]. This outcome suggests that the drug doses administered were appropriate to prevent respiratory depression, as this effect is

known to be dose-dependent. The absence of interdigital reflex and adequate muscle relaxation observed in all animals indicates effective sedation and analgesia, essential for conducting clinical assessments without causing distress or compromising safety. Movements observed in some animals may suggest varying degrees of sedation depth or individual responsiveness to the anesthetic agents [5].

The occurrence of an arrhythmia in one animal underscores the importance of electrocardiographic monitoring during anesthesia. Opioids, such as morphine, can predispose animals to cardiac dysrhythmias. Managing arrhythmias promptly with reversal agents like naloxone highlights the critical role of vigilant monitoring and preparedness for adverse events during anesthesia [11].

Furthermore, implementing anesthetic protocols that include reversal agents for multiple drugs is crucial to ensure the safety and welfare of wild animals during clinical procedures. The ability to reverse the effects of drugs allows for a quicker and smoother recovery of the animals, reducing the risk of post-anesthetic complications. This approach also provides an additional layer of safety by allowing for immediate correction of overdoses or adverse reactions, minimizing the potential mortality associated with anesthesia in wild species [12]. As evidenced in this study, naloxone successfully reversed the arrhythmia, underscoring the efficacy of reversal agents in addressing anesthesia related complications.

Anesthesia recovery is a critical period in the chemical restraint of wild cats, and a short recovery period is recommended for both captive and free-living animals [13]. Due to the variation in anesthetic recovery depending on the drug used, species, duration of anesthesia and individual variations, the use of pharmacological antagonists offers distinct advantages [5]. Researchers observed the effects of yohimbine in reversing the restraint of lions, tigers and leopards, achieving a smooth anesthetic recovery without excitatory events [14] 12. In the present study, all the animals received the combination of naloxone and yohimbine resulting in fast and peaceful recovery without adverse effects.

Most of animals exhibited smooth anesthesia recovery, indicating the overall effectiveness of the anesthesia protocols and reversal agents. However, cases requiring additional ketamine dosing experienced exacerbated adverse effects during recovery, such as increased salivation, muscle tremors and ataxia, influencing the quality of anesthetic recovery.

Overall, this study contributes valuable insights into the anesthesia management of Southern Tiger Cats (*Leopardus guttulus*), highlighting the importance of tailored protocols, vigilant monitoring, and prompt intervention in optimizing anesthesia outcomes and ensuring animal welfare. Further research is warranted to explore alternative anesthetic regimens, refine reversal strategies, and enhance anesthesia safety for wildlife species in conservation and veterinary care settings.

CONCLUSION

Both the morphine-xylazine-ketamine and methadone-xylazine-ketamine protocols showed comparable efficacy in Southern Tiger Cats (Leopardus guttulus), providing stability in the physiological parameters monitored. The administration of antagonists further facilitated smooth recovery, providing valuable insights into anesthetic protocols for endangered species.

Data Availability Statement

The data that support the findings of this study are not openly available due to reasons of sensitivity and are available from the corresponding author upon reasonable request. Data are located in controlled access data storage at University of the Southern Frontier.

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Authors' contributions

JCB, MM, ALRM, and FLCG participated in study design, data management, manuscript preparation, statistical analysis, data interpretation, and data acquisition. ZSC contributed to data management, statistical analysis, data interpretation, and data acquisition. GFG and FD were involved in data management, data interpretation, and data acquisition. TC and GCF made contributions to study design, data management, manuscript preparation, statistical analysis, data acquisition, and supervision.

Declaration of conflicting interests

The authors have no competing interests to declare and declare that this research was conducted with respect during its execution and publication of this article

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HEMIJSKO SEDIRANJE TIGRASTE MAČKE (*LEOPARDUS GUTTULUS*) METADONOM ILI MORFINOM SA KSILAZIN-KETAMINOM

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Južna tigrasta mačka *(Leopardus guttulus*) je ugrožena vrsta kojoj je potrebna efikasna anestezija za kliničke zahvate, ali nedostaju studije specifične za ovu vrstu. Ova studija ima za cilj procenu i upoređivanje efikasnosti metadona ili morfina u kombinaciji sa ksilazin-ketaminom za imobilizaciju južnih tigrastih mačaka (*Leopardus guttulus*). Životinje su nasumično raspoređene da prime sedaciju metadonom (0,3 mg/kg) ili morfinom (0,5 mg/kg) u kombinaciji sa ksilazin-ketaminom (1,0 mg/kg i 10,0 mg/kg), pri čemu su preparati aplikovani intramuskularno. Parametri elektrokardiografije i fiziološke varijable su beleženi svakih pet minuta od momenta primene. Nakon 40 minuta procene, primenjen je protokol za reverziju opioida i α2-adrenergičkog agonista koristeći nalokson i johimbin, i kvalitet oporavka je evaluiran. U ovoj studiji nisu primećene značajne razlike između grupa ili vremena procene u fiziološkim varijablama koje su praćene. Jedan subjekt je pokazao idioventrikularni ubrzani ritam, što je pripisano primeni morfina i potvrđeno njegovom reverzijom. Evaluacija oporavka pokazala je povoljne ishode, iako su neke životinje pokazale neželjene efekte, pogoršane dodatnim dozama ketamina. Protokoli morfin-ksilazin-ketamin i metadon-ksilazin-ketamin pokazali su sličnu efikasnost kod južnih tigrastih mačaka *(Leopardus guttulus*), sa stabilnim fiziološkim parametrima i kvalitetnim oporavkom. Upotreba antagonista omogućila je nesmetan oporavak, doprinoseći vrednim uvidima u anesteziološke protokole za ugrožene vrste. Primenjeni protokoli obezbedili su siguran pregled i vredan uvid u anesteziološke protokole za ovu ugroženu vrstu.