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Short Communication

Standardization of anesthetic dose for immobilization of captive-bred Red Panda in Padmaja Naidu Himalayan Zoological Park, Darjeeling, West Bengal, India

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ABSTRACT

The present study was done to get a standard dose of xylazine/ketamine with minimum recovery time. Ten healthy red pandas were anesthetized for the blood collection for their blood biochemistry and hematological studies at the Padmaja Naidu Himalayan Zoological Park, Darjeeling. The animals were administered with xylazine/ketamine according to their body weight and recovery time was calculated. We found that immobilization was successful with a low dose of ketamine which was found very low as compared to other authors. The lower anesthetic dose was effective in young animals but there was no relation between age and recovery time. Based on the findings of the present study we can conclude that red pandas between 4-7 kg body weight could be easily immobile with the dose of xylazine/ketamine between 2-5 mg/kg and can be recovered in 25 minutes. The conclusion is completely based on the study of the captive-bred red pandas in the Padmaja Naidu Himalayan Zoological Park. The results of this study may vary from other zoos due to geographical and regional differences.

Key words: Red panda, immobilization, xylazine, ketamine, recovery time

INTRODUCTION

It has always been a challenging task to immobilize wild animals and, there is limited information regarding the anesthetic protocol to be used in both captive and wild. Many complications are encountered due to the vast variations in estimating the actual dose according to the body weight, age, and gender. To conclude exact recovery time with an immobilizing dose is also challenging.

There are three methods applied for immobilization purposes: tranquilization, sedation, and analgesia. Tranquilization produces calmness, loss of aggression, and loss of alertness without drowsiness. Drug-induced sedation has a more intense effect and produces sleepiness and hypnosis. Analgesia is the reduction of pain, which works according to a drug's effect. Many drugs cannot be classified by only one pharmacologic effect, i.e., tranquilizers, sedatives, or analgesics (Linda, 2015). For example, many psychotropic drugs can either tranquilize or sedate according to the dose administered, and many sedatives are also analgesics. Also, drugs classified as tranquilizers, sedatives, and/or analgesics may have extra effects like behavioral changes (Linda, 2015).

The drugs used to tranquilize animals are Telazol, Acepromazine, Diazepam, Xylazine, Medetomidine, and Azaperone. Ketamine, Tiletamine, Etorphine are dissociative anesthetic which is also called Cyclohexane and always used in combination with sedatives or tranquilizers.

Xylazine hydrochloride is used for sedative and tranquilizing purposes in a variety of species. Ketamine hydrochloride is the most used injectable anesthetic used in a variety of species. For the present study, we selected xylazine and ketamine. However, Schaftenaar (1993) and Janno (2015) used medetomidine in combination with ketamine for red panda immobilization (Table 1). Before administration xylazine/ketamine can be mixed in a single syringe. This is the most common injectable anesthetic combination used in red pandas (Edwards, 2012; Jha *et al.,* 2014; Wolf *et al.,* 1990) (Table 1) and other animals (Flecknell, 1987; Gaertner et al., 2008; Green *et al.,* 1981; Richardson & Flecknell, 2005). The joint synergetic effect of tranquilizer and anesthetic is extremely better than the individual effect of either of the two drugs for smooth initiation, relaxed immobilization, and softer recovery. Ketamine alone does not give muscle relaxation and muscle cramps may be seen. The modern repertory of drugs such as medetomidine, tiletamine, zolazepam, and atipamezole is not available in India, whereas xylazine hydrochloride and ketamine hydrochloride are readily available (Belsare & Athreya, 2010).

As far as we inspected, we found that there is no any information available about the standard dose of immobilization with the limited recovery time of red panda from any zoo. Therefore, it was needed to execute the present study. This paper aims at presenting a standard dose of immobilizing captive red pandas with a combination of xylazine hydrochloride and ketamine hydrochloride. The whole study was done in red panda

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Table 1. List of Chemical immobilization in Red Panda by different authors

Dosage (mg/kg) and combination	Authors
Xylazine (0.2–0.4) and Ketamine (10–15)	Edward, 2012
Ketamine (6.6) and Medetomidine (0.080)	Schaftenaar, 1993
Ketamine (5) and Medetomidine (50µg/kg)	Janno, 2015
Xylazine (0.2-0.4) and Ketamine (5-10)	Jha *et al.,* 2014
Ketamine (6–9) and Xylazine (0.2–0.4)	Wolf *et al.,* 1990

(*Ailurus fulgens fulgens*), a critically endangered species. In the context of the declining population of the red panda in the wild (Glatston *et al.,* 2015; Bista et al., 2017), animals kept in the zoo should be physically fit as a stock population for captive breeding and reintroduction programs. The present work was also important in the concern of the health study of captive bred red pandas.

Factor affecting anesthesia response:

According to the author Sontakke *et al.,* 2017, some factors play important role in the anesthesia process. These factors are age, sex, size, body weight, species, physical condition, pregnancy, and seasons. Sontakke *et al.,* 2017 mentioned that young and much older animals are more sensitive to anesthesia as compared to adult animals. Young and short animals require higher doses than older and tall animals. The authors also said that males need higher doses than females. Additionally, pregnant animals require more doses. Sick and weak animals can be anesthetized with a lower dose than healthy animals. Seasons have a deep effect on response to some anesthetic drugs (Sontakke *et al.,* 2017).

MATERIAL AND METHODS

Animal subjects

Red pandas selected in this study were from the Padmaja Naidu Himalayan Zoological Park, Darjeeling, dedicated to research designed for studying the effect of xylazine/ ketamine. A total of ten healthy pandas were involved in the whole study. The total population of red pandas in the zoo is twenty-two. Therefore, the sample size of the present study is according to the method called the "resource equation" method (Charan & Kantharia, 2013; Festing, 2006; Festing & Altman, 2002).

Chemicals

Xylazine hydrochloride (100mg/ml) and Ketamine hydrochloride (50mg/ml) both were bought from the chemical shops under the guidance of the zoo veterinary doctor. Xylazine hydrochloride is chemically designated N-(2,6-dimethyl phenyl)-5,6-dihydro-4H-1,3-thiazin-2-amine; hydrochloride ($C_{12}H_{17}ClN_2S$). Ketamine hydrochloride is chemically designated 2-(2-chlorophenyl)-2-(methylamino)cyclohexan-1; hydrochloride ($C_{13}H_{17}C_{l2}NO$).

Treatments

Pre-anesthetic preparations smooth the procedure and increase patient safety. Food and water were withheld for 12 hours to minimize vomiting. Animals were caught in nets, weighed, and an anesthetic mixture was administered. A needle length of 35 mm was used for intramuscular injection. The preferred sites for injection were the femoral thigh muscle. The anesthesia was given by the zoo veterinary doctor. During anesthesia body temperature, respiratory rate, heart rate, blood pressure, and eye reflexes were monitored.

We chose not to use the prescribed xylazine (50mg/kg) and ketamine (100mg/kg) ratio (Vyas, 2016) because it was a high dose for the red panda. After xylazine/ketamine injection, recovery time was measured as the time from the first voluntary movement until full movement observed. Details of treatment are mentioned in (Table 2).

Table 2. Details of treatment and doses of Xylazine & Ketamine administered in captive red pandas (n=10)

House Names	Age (Years/Months)	Gender (Female/Male)	Bodyweight (Kg)	Total dose administered		Recovery Time (Hours/ Minutes)
				Xylazine (mg/Kg)	Ketamine (mg/Kg)	
NIKKI	2Y8M	F	5.315	3.763	2.822	1h23m
RAM	12Y8M	M	4.720	4.237	3.178	32m
SHIFU	6Y11M	M	5.630	3.552	2.6643	47m
BALAM	5Y9M	M	5.750	5.217	3.478	20m
NOEL	5Y8M	M	5.215	5.753	3.835	34m
PABU	3Y7M	M	5.050	3.960	2.97	38m
KIMBU	2Y8M	M	6.410	4.680	3.9	17m
KARMA	10Y9M	F	6.083	4.932	3.288	1h
PRASENJIT	1Y9M	M	6.400	3.125	3.906	14m
SATVIK	1Y10M	M	6.739	2.944	5.152	48m

RESULTS

The results showed that there was a strong positive and significant (p 0.01) correlation between body weight and ketamine doses (Table 3). This means that the dose of ketamine was increasing with the body weight of the red panda (Figure 1). A small positive correlation was observed between body weight and xylazine, but this relation was not significant (Table 3, Figure 1).

The body weight and recovery time showed a small negative relationship. It means that when body weight was increasing, recovery time was decreasing. There was no significant correlation observed between body weight and recovery time (Table 3, Figure 2).

A large negative correlation was observed between age and ketamine dose, while xylazine showed a small positive relation. This result says that when the age of red panda was increasing the dose of ketamine was decreasing. But opposite to ketamine, xylazine dose was increasing with the age of red panda (Table 3, Figure 3). This relationship was not significant.

The relationship between age and recovery time indicates that while both tend to go up in response to one another, the relationship was not very strong. This result was also not significantly correlated to each other (Table 3, Figure 4).

A small negative correlation between xylazine/ketamine and recovery time was observed. This defines that when xylazine/ketamine dose was increasing the recovery time was decreasing. There was no significant relationship observed (Table 3, Figure 5).

Table 3. Pearson correlation analysis of tranquilization study in captive red pandas (n=10)

	Body Weight	Age	Xylazine	Ketamine	Recovery time
Body Weight	–		.163	.877**	-.185
Age		–	.179	-.519	.092
Xylazine			–		-.265
Ketamine				–	-.249
Recovery time					–

** Correlation is significant at the 0.01 level (2-tailed)

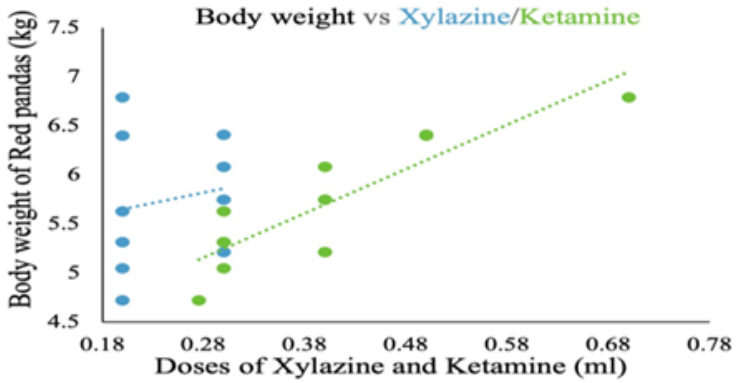

Figure 1. Bodyweight shows a strong positive correlation with Ketamine and a small positive correlation with Xylazine

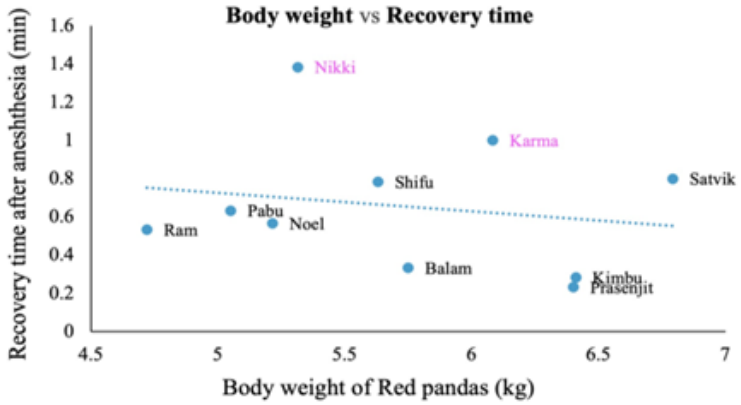

Figure 2. Represents small negative correlation between body weight and recovery time

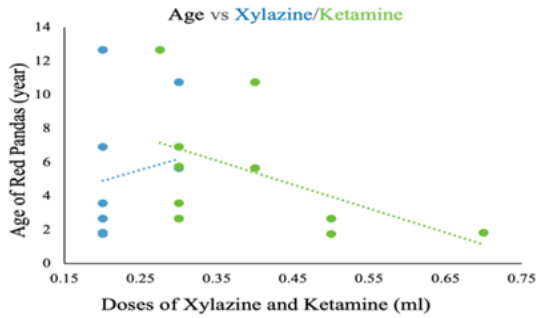

Figure 3. Conveys small positive and large negative correlation between age and xylazine/ketamine respectively

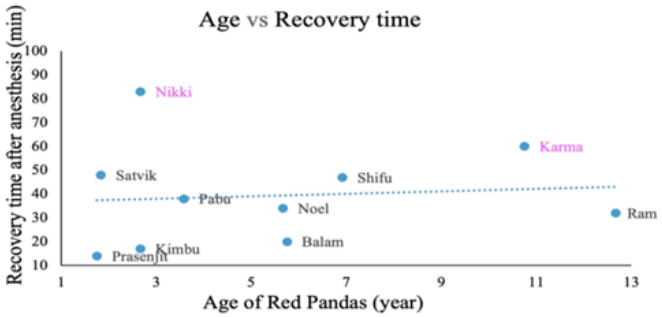

Figure 4. Shows the weak positive correlation between age and recovery time

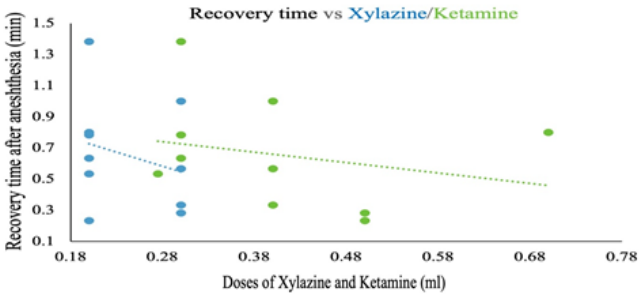

Figure 5. Represents small negative correlation between recovery time and xylazine/ketamine

DISCUSSION

There is very little literature available on the tranquilization of the red panda. The present study was done to get a standardized dose of xylazine/ketamine for the tranquilization of the captive-bred red panda at Padmaja Naidu Himalayan Zoological Park, Darjeeling. During the whole study, we selected xylazine/ketamine dose between 2-5 mg/kg per body weight of red panda (Table 2). This dose was less in comparison to other authors (Edwards, 2012; Jha *et al.,* 2014; Wolf *et al.,* 1990) (Table 1 and Table 2).

We also tried to find out an optimum recovery time with this dose. In the present study, we found 25.223 ± 5.16 SE minute recovery time. According to us this (25 min) is enough time for blood collection from the red panda to perform the hematological and biochemical study. However, none of any information regarding recovery time for red pandas has been reported by any author. Based on our study, we can recommend that xylazine/ketamine is a good combination to get 25 minutes of recovery time in red pandas. According to Schaftenaar (1993) and Janno (2015) the best choice of injectable anesthetic agents for the red panda is ketamine in combination with medetomidine (Table 1). This combination is completely different from our study. A comparative study of medetomidine/ketamine and xylazine/ketamine has indicated that the recovery from medetomidine/ketamine took longer than recovery from xylazine/ketamine (Moens & Fargetton, 1990). However, this study was done on dogs.

Other drug combinations have also been used in juvenile and adult red pandas i.e., ketamine (10–15 mg/kg) with xylazine and followed by diazepam (0.2–0.5 mg/kg); and tiletamine/zolazepam (4.5–6.0 mg/kg) (Wolff *et al.,* 1990). The popularity of xylazine/ketamine is mainly due to its supplemental effects (that is, analgesic properties, muscle relaxation, and sedation) (Ullah, 2017; Wyatt *et al.,* 1989).

In the current study, we do not find a significant correlation between body weight, xylazine dose, and recovery time. But the bodyweight and ketamine showed a significant positive correlation. Although the dose of ketamine was increasing with the increase in body weight of animals. But the dose (2-5 mg/kg) of ketamine was very lower than other authors (Table 1). This shows that ketamine plays important role in animal

anesthesia, and it works nicely with low doses (2-5 mg/kg) in red panda. According to Wolff *et al.,* (1990) the dose of xylazine and ketamine should be (0.2-0.4 mg/kg) and (6-9 mg/kg) respectively (Table 1). Jha *et al.,* (2014) suggested that the doses should be between 0.2-0.4 mg/kg of xylazine and between 5-10 mg/kg of ketamine while Edwards (2012) said that xylazine should be (0.2-0.4 mg/kg) and ketamine (10-15 mg/kg) (Table 1). The dose of ketamine suggested by Edwards, 2012; Jha *et al.,* 2014; Wolf *et al.,* 1990 was also very higher than the dose taken in our study. Effects of low and high doses of ketamine have also been studied by some authors whose results support the finding of the present study (Ambros & Duke, 2013; Aronson, 2007). Though Verstegen *et al.,* 1990 study was comparative between medetomidine/ketamine and xylazine/ketamine, he also found that a lower dose of ketamine was longer in the duration of action and better analgesia. According to Aroson (2007) low-dose ketamine may avoid adverse effects while providing high-quality analgesia. Giroux *et al.,* 2015 said that a high dose of xylazine/ketamine can increase the anesthesia duration. Additionally, Dematteis *et al.,* 2006 have also reported a quick and smooth recovery time with a low dose of xylazine/ketamine in the Himalayan Tahr. Although the dose of xylazine was higher in the present study than in other studies (Table1 and Table 2), the presence of a negative relationship between the xylazine dose and recovery time suggests that increasing the dose did not expand the effectiveness of immobilization.

The relationship between age and anesthetic dose was not significantly correlated but results showed that younger animals required a higher dose of xylazine while a lower dose of ketamine for immobilization. As in the present study, the xylazine dose was almost common at every age of animals. We can say that this result does not follow the statement of Sontakke *et al.,* 2017 who said that younger animals can be tranquilized with a higher dose than old ones. Moreover, we find a weak insignificant positive relationship between age and recovery time. Therefore, the lack of a strong relationship between age and recovery time suggests that anesthetic doses do not affect age. However, Giroux *et al.,* 2015 found that recovery time increases with the age in rats. Most female pandas were during the gestation and maternity period. Because of this, there was some constraint to include more females in the present study. But Figures 2 and 4 showing that females required more recovery time than male red pandas while Olatunji *et al.,* 2016 have reported just the reverse where male rats needed more recovery time than female rats. It might be possible in the present study this difference was due to the higher dose required by males. There are species-specificity and variations in drug response (Sontakke et al., 2017). Further, a gender difference study is required to conclude this.

CONCLUSION

Although there are many variations found from literatures in doses of xylazine/ketamine, based on our study we can suggest that the best dose of xylazine/ketamine should be (2-5mg/kg). This dose is best choice to get less recovery time for small medical purposes in red pandas. The present study was completely conducted on red pandas of Padmaja Naidu Himalayan Zoological Park, Darjeeling. Thus, author concluding their results might differ than the other zoos. This difference might be possible due to ecological and environmental differences. Further, comparative region-based studies should also be done.

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CONFLICTS OF INTEREST

The authors have indicated that they have no other conflicts of interest regarding the content of this article.

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