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RESEARCH ARTICLE

# Comparative Efficacy of Yohimbine and Tolazoline for Antagonism of Ketamine-Xylazine Induced Sedation in Captive Wild Felids

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## Abstract

This study was conducted to investigate the comparative efficacy of yohimbine and tolazoline for antagonism of ketamine-xylazine induced sedation in captive wild felids in Pakistan. It included 16 tigers (Panthera tigris), 22 lions (Panthera leo) and 16 leopards (Panthera pardus), aged between 2-10 years, weighing approximately 190.6±12.4 kg, 161.6±16.6 kg, and 50.5±6.9 kg respectively. A total of 54 anesthetic inductions were carried out on clinical patients from all species dividing them into two groups KX-T and KX-Y receiving 0.15 mg/kg tolazoline and 0.15 mg/kg yohimbine as antagonists, respectively. Body temperature, pulse and respiration rate (TPR) were recorded at ten-minute intervals for thirty minutes in either groups. These physiological norms differed significantly only in Tigers at 10min and 20min intervals. Furthermore, onset of arousal and recovery time in animals receiving yohimbine was evidently shorter, ranging between 2.8±0.76 to 8.42±0.33 min. ALT, AST, Urea and Creatinine were significantly elevated in groups administered with Tolazoline when compared to Yohimbine as well. Hence, yohimbine at described dosage effectively antagonized ketamine and xylazine anesthesia by significant reduction in reversal times for all the species under consideration, bearing nominal deleterious effects on physiological, hepatic and renal parameters. So, this study concludes yohimbine to be a superior antagonist for ketamine-xylazine anesthetic reversal than tolazoline.

***Keywords:*** *Leopard, Lion, Reversal, Tiger, Tolazoline, Yohimbine*

# Kafeste Tutulan Yabani Kedigillerde Ketamin-Ksilazin İle İndüklenen Sedasyon Antagonizmi İçin Yohimbin ve Tolazolinin Karşılaştırmalı Etkinliği

## Öz

Bu çalışma, Pakistan'da kafeste tutulan yabani kedigillerde ketamin-ksilazin kaynaklı sedasyon antagonizmi için yohimbin ve tolazolinin karşılaştırmalı etkinliğinin araştırılması için yapıldı. Çalışmada, yaşları 2 ile 10 arası değişen 190.6±12.4 kg ağırlığında 16 kaplan (Panthera tigris), 161.6±16.6 kg ağırlığında 22 aslan (Panthera leo) ve 50.5±6.9 kg ağırlığında 16 leopar (Panthera pardus) yer aldı. Tüm hayvan türlerinden klinik hastalara toplam 54 anestezik indüksiyonu gerçekleştirildi ve antagonist olarak sırasıyla 0.15 mg/kg tolazolin ve 0.15 mg/kg yohimbin verilerek KX-T ve KX-Y adlı iki gruba ayrıldı. Her iki gruptaki hayvanların vücut ısısı, nabız ve solunum sayıları (TPR) on dakika aralıklarla otuz dakika süreyle kaydedildi. Bu fizyolojik normlar, yalnızca kaplanlarda 10. dakika ve 20. dakikada önemli ölçüde farklılık gösterdi. Ayrıca, yohimbin uygulanan hayvanlarda ayılma ve derlenme süresinin başlaması, 2.8±0.76 ile 8.42±0.33 dakika ile belirgin olarak daha kısaydı. Tolazolin uygulanan grupta ALT, AST, üre ve kreatinin seviyesi, yohimbin ile karşılaştırıldığında önemli ölçüde daha yüksekti. Dolayısı ile, bildirilen dozda yohimbin kullanımı, fizyolojik, hepatik ve renal parametreler üzerinde önemsiz zararlı etkileri ile söz konusu tüm hayvan türlerinin anesteziden uyanma sürelerinde önemli bir azalma sağlayarak ketamin ve ksilazin anestezisini etkili bir şekilde antagonize etti. Bu nedenle bu çalışmada, ketamin-ksilazin anestezisinden uyanmada yohimbinin, tolazolinden daha üstün bir antagonist olduğu sonucuna varıldı.

***Anahtar sözcükler:*** *Aslan, Kaplan, Leopar, Tolazolin, Uyanma, Yohimbin*

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## Introduction

Lions, tigers and leopards have been classified as vulnerable or endangered species on the International Union for Conservation of Nature (IUCN) Red List, forecasting a dreary future for their survival [1]. These days, most of the lions and wild tigers live in sparsely and sporadically distributed populaces, thereby increasing the probability of inbreeding [2-4]. Unlike tigers and lions, leopards are indigenous to varied regions in the Indian subcontinent [5]. They were once seen across almost all terrains of Pakistan. But these days, Indian subspecies of *Panthera pardus* may only be seen running wild around metropolitan edges of Islamabad to certain mountain ranges in the north [6]. Considering such a scenario, the survival of wild carnivorous felids largely depends upon captive breeding in zoos and wildlife reserves. Therefore, it has become imperative that scientific inquiries are undertaken to gain information about physiology, pathology and treatment of diseases that afflict these species [7]. Sedative and fast-acting anesthetics are pre-requisites in the field of wildlife medicine [8]. Their use has become ever more frequent in the last five decades [9]. Research and management of feral species require a reliable immobilization protocol that can easily be reversed and poses the least amount of hazard to the physiology of that animal [10]. Captive felids regularly need chemical restraining or tranquilization to assist in clinical examination, vaccination, sample collection, administration of medication and minor surgeries [11]. These animals are generally tranquilized using remote delivery devices such as pole syringes or darts. Monitoring of animals in tranquilized state is usually not possible in field settings so the drugs used must have a broad safety margin and must not incur resounding deleterious effects on vital parameters [12]. Ketamine-xylazine tranquilization is widely practiced in zoo settings and has been used effectively to immobilize several wild felids including lions, leopards and tigers [13]. The sedative and relaxing effects of xylazine counteract pressor and cataleptic effects of ketamine [14,15]. Nevertheless, alpha-2 agonists have been observed to cause bradycardia, ventricular arrhythmias, hypertension and hypotension in tigers and leopards [16,17]. Consequently, a suitable antagonist for speedy recovery of tranquilized patient is greatly needed to avoid cardiovascular instability [17]. Both yohimbine and tolazoline are alpha-2 adrenergic antagonists [9]. Tolazoline is capable of ameliorating respiratory depression and muscle relaxation attributed to xylazine [9,18,19]. Upon recovery, a marked lack of co-ordination may also be resolved by tolazoline administration [20]. Yohimbine, similar to tolazoline has been reported to significantly shorten recovery from sedation [21]. Effects of alpha-2 adrenergic antagonists such as atipamezole, yohimbine, and tolazoline have been extensively studied in ruminants to antagonize anesthetic effects of ketamine-xylazine combination [21,22]. Moreover, studies in domestic cats, lions, Bengal tigers and leopards have proposed that yohimbine and tolazoline could accelerate the recovery from ketamine-xylazine anesthesia [23]. The present study was undertaken to compare the ability of yohimbine and tolazoline in antagonizing the anesthetic effects of ketamine-xylazine in three endangered large felids (lions, leopards and tigers).

## Material and Methods

### *Selection of Animals*

This study included wild felids that had to be anesthetized for clinical or minor surgical procedures. All captive tigers *(Panthera tigris),* leopards *(Panthera pardus)* and lions *(Panthera leo)* included in this study were male, aged between 2-10 years. The study was undertaken during years 2018 and 2019 at day and night zoo, Bahria town Karachi and amongst some privately kept animals at farm-houses around the adjoining areas of Lahore. A total of 54 anesthetic inductions were carried out on 16 male tigers, 22 male lions and 16 male leopards *(Table 1)*. The mean body weight (mean ± SD) of these wild felids were; male tigers 190.6±12.4 kg (range 173.6-210 kg), male lions 161.6±16.6 kg (range 134.3-189.2 kg), and male leopards 50.5±6.9 kg (range 42.6-64.2 kg) respectively. All animals selected for this study were adequately de-wormed and vaccinated previously. Nutritional requirements were fulfilled by beef along with weekly calcium and vitamin supplementation. Wild felids inducted into this study were allowed to consume water freely during day or night. This study included animals that had to be sedated for clinical or managerial functions at privately owned zoos and farm houses in Pakistan. All procedures were conducted in line with prescribed guidelines of "The prevention of cruelty to animals act, 1890" and "The Punjab wildlife act, 1974" of Pakistan.

### *Anesthetic Procedure*

All animals were kept off feed 8 h before the anesthetic procedure, to avoid any complications. All animals were anesthetized in the early hours of the day, between 09:00-12:00 hours with a combination of 2.6 mg/kg ketamine HCl (Ketarol® 50 mg/mL, Global Pharmaceuticals) and 1.3 mg/kg xylazine HCl (Xylaz® 20 mg/mL, Farvet). The combination of anesthetic drugs was loaded into a 5ml dart syringe equipped with a 35-mm needle. Thigh or the shoulder region were targeted using a blowpipe to inject these drugs intramuscularly with a plastic projectile dart. We preferred using projectile darts as opposed to squeeze cages, because wild felids had to be lured with

***Table 1.*** *Number of tigers, leopards and lions involved in anesthetic trial*

| Zoo | Tigers | Leopards | Lions |
|---|---|---|---|
| | Male | Male | Male |
| Danzoo, Bahria town Karachi | 9 | 10 | 14 |
| Privately kept wild felids at farm houses around Lahore | 7 | 6 | 8 |

food to go into them. However, to avoid any iatrogenic problems, animals had to be kept off-feed 6-8 h before tranquilization when xylazine has to be employed. As xylazine is commonly associated with emesis in felines if the animal has fed recently [24]. Therefore, feeding them immediately prior to anesthetic administration might have caused drenching of vomitus during transportation or recumbency. After the animals were darted, they were left undisturbed and were monitored from an adequately safe distance until they showed signs of anesthetic induction. Anesthetic induction time was judged by oropharyngeal tone, sedation, incoordination and pupillary dilation. After being completely anesthetized, the feline species were moved on a stretcher in lateral recumbency to another cage for the required procedure. To avoid any iatrogenic problems, ophthalmic ointments were administered on cornea and conjunctiva as anesthetized cats lose control of their palpebral muscles. Furthermore, while transportation their eyes were covered to protect them from bright sunlight. It was observed by Sontakke et al.[20] that duration of immobilization in wild felids can range up to 31-40 min after a single loading dose of xylazine and ketamine combination. A maintenance dose of ketamine and xylazine was not required to prolong anesthesia. The overall duration of anesthesia included the duration of induction and immobilization similar to prior investigations. Parameters, such as pulse, rectal temperature and respiratory rates were monitored immediately after anesthetic induction at every 10-min interval until anesthetic recovery.

### *Reversal of Anesthesia*

The procedures performed on subjects were minor and mostly diagnostic in nature therefore did not take more than 15 min. Consequently, all wild felids were administered with either yohimbine or tolazoline irrespective of the species differences 20 min after induction of anesthesia. Within each species, anesthetized animals were divided into two groups: Group KX-T received an intravenous injection of 0.15 mg/kg tolazoline (Tolazine®, Akorn Animal Health) while the second group namely KX-Y received an intravenous injection of 0.15 mg/kg Yohimbine (Antagozil®, Troy Laboratories). The time duration that elapsed from the point of reversal administration (tolazoline/yohimbine) to the animal being able to stand was deemed as Standing/ Recovery time. Individuals were monitored for up to 12 h after recovery to identify any signs of stupor or sedation.

### *Effect of Anesthesia on Liver and Renal Function*

One day before the procedure each animal was restrained in a squeeze cage and phlebotomy was performed via the coccygeal route for blood sample collection. The blood sample was transferred from the syringe to the serum gel vacutainer and labeled appropriately. The values of liver function test (LFT) and renal function test (RFT) obtained from this blood sample, established the baseline values. A second sample of blood was collected from individuals in both groups (i.e., tolazoline and yohimbine) 12 h after the completion of the procedure for serum analysis. Liver function test (LFT) included evaluation of aspartate amino-transferase (AST) and alanine transaminase (ALT) while renal function test (RFT) involved biochemical assessment of blood serum concentrations for urea and creatinine.

### *Ethical Considerations*

This study was conducted under the proposed guidelines outlined by Pakistan's Prevention of Cruelty to Animals Act (1890) and is in compliance with the Guide for the Care and Use of Agricultural Animals in Research and Teaching.

### *Statistical Analysis*

All data analyses were performed using GraphPad Prism (Ver. 8.4.3 for Windows; GraphPad Software, La Jolla California, USA). Data are presented as mean ± SD. An unpaired t-test analyzed the efficacy of two drugs as reversal agents against anesthesia. One-way ANOVA followed by a Tukey test was used for evaluation of blood chemistry parameters while two-way ANOVA analysis followed by the Sidak test was employed to compare the effects of two drugs on respiration, pulse and body temperature at different time intervals. A probability of $P<0.05$ was considered to be the minimum level of significance.

## Results

### *Induction of Anesthesia*

Anesthetic induction using a combination of 2.6 mg/kg ketamine and 1.3 mg/kg xylazine produced effective sedation. Loss of muscular tone, pupillary dilatation and absence of oropharyngeal reflex within twenty minutes of darting indicated adequate analgesia and deep sedation. As ataxia started to set in, and the animal became recumbent laterally, the time of anesthetic induction was tallied. The induction times in the tiger and lion were comparable but leopards took a significantly shorter period of time to get sedated *(Table 2).* Ear twitch and palpebral reflexes were checked to monitor the depth of anesthesia. The animals involved in this study were anesthetized either to transfer them from one site to another or to perform wound management. The procedures were thereafter completed and animals were transferred to their housing pens for recovery from anesthesia.

***Table 2.*** *Time to induction of anesthesia (minutes) in the captive tigers, lions and leopards*

| Sr. No | Specie | Gender | Number of Animals | Induction Time |
|---|---|---|---|---|
| 1 | Tiger | Male | 16 | 18.17±1.177[a] |
| 2 | Lion | Male | 22 | 18.39±1.8[3]a |
| 3 | Leopard | Male | 16 | 13.93±1.1[3]b |

*Values are represented as mean ± SD. Different superscripts in a column within a species differ significantly whereby P<0.05*

***Table 3.*** *Average respiration rates (breaths per min), average pulse rates (beats per min) and average body temperature (°F) at different time intervals for male tigers, lions and leopards during immobilization administered with Tolazoline and Yohimbine*

| Specie | Antagonist | Average Respiration Rates at Different Time Durations | | | Average Pulse Rates at Different Time Durations | | | Average Body Temperature at Different Time Durations | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 10 | 20 | 30 | 10 | 20 | 30 | 10 | 20 | 30 |
| Tiger | Tolazoline | 18.87±1.45$^a$ | 20±1.41$^a$ | 20.5±2.56$^a$ | 67.5±3.16$^a$ | 59.25±2.5$^a$ | 52.8±1.6$^a$ | 102.4±0.2$^a$ | 101.85±0.24$^a$ | 101.05±0.24$^a$ |
| | Yohimbine | 23.125±2.47$^b$ | 23.5±2.67$^b$ | 21.625±2.5$^a$ | 58.75±1.6$^b$ | 72.5±1.19$^b$ | 65.8±2.58$^b$ | 102.35±0.24$^a$ | 104.2±0.48$^b$ | 103.05±0.24$^b$ |
| Lion | Tolazoline | 20.45±3.32$^a$ | 21.90±4.06$^a$ | 21.54±2.16$^a$ | 65.81±3.31$^a$ | 57±3.84$^a$ | 53.45±2.06$^a$ | 102.50±0.34$^a$ | 102±0.33$^a$ | 101.2±0.33$^a$ |
| | Yohimbine | 22.63±1.91$^a$ | 22.45±2.50$^a$ | 21±2.04$^a$ | 62.5±6.12$^a$ | 70.6±2.73$^b$ | 65.63±4.08$^b$ | 101.43±1.03$^b$ | 102.61±1.81$^a$ | 102.25±0.92$^b$ |
| Leopard | Tolazoline | 19.62±1.30$^a$ | 20.62±2.06$^a$ | 21.37±1.99$^a$ | 71.5± 2$^a$ | 67.62±4.47$^a$ | 64.62±6.69$^a$ | 101.66±0.88$^a$ | 101.16±0.84$^a$ | 100.55±0.70$^a$ |
| | Yohimbine | 22.5±2.20$^b$ | 21.12±2.9$^a$ | 19.25±1.48$^a$ | 67±3.54$^b$ | 72.12±1.12$^a$ | 69.37±2.82$^a$ | 101.66±0.33$^a$ | 102.52±0.83$^b$ | 102.21±0.41$^b$ |

*Values are represented as mean ± SD. Different superscripts in a column within a species differ significantly whereby P<0.05*

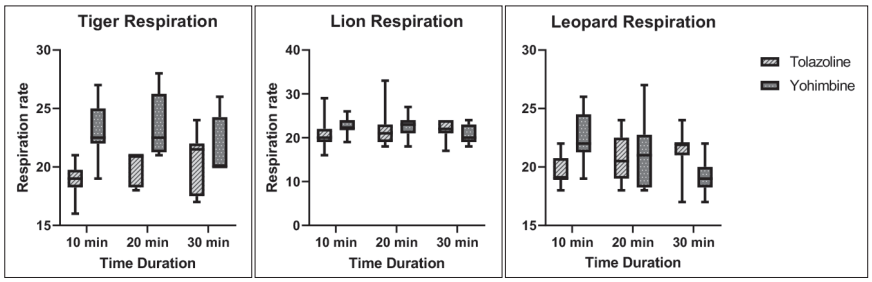


**Fig 1.** Interleaved Box and Whisker plots (GraphPad Prism Ver. 8.4.3) depicting comparative respiratory rates after tolazoline and yohimbine administration in Tiger, Lion and Leopard immobilized with ketamine-xylazine anesthesia at 10 min, 20 min, 30 min time intervals

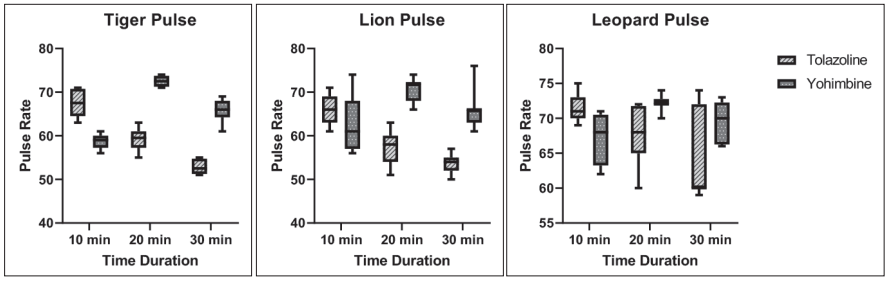


**Fig 2.** Interleaved Box and Whisker plots (GraphPad Prism Ver. 8.4.3) depicting comparative Pulse rates after tolazoline and yohimbine administration in Tiger, Lion and Leopard immobilized with ketamine-xylazine anesthesia at 10 min, 20 min, 30 min time intervals

### *Values for Physiological Norms*

As reversal was injected in either group receiving tolazoline or yohimbine intravenously, physiological parameters such as respiration, pulse and body temperature were recorded at 10 min intervals for a period of thirty minutes *(Table 3)*.

In tigers, respiration rates differed significantly at 10 min (P=0.0014) and 20 min (P=0.0098) post tolazoline or yohimbine administration, however, the difference became insignificant by 30$^{th}$ min (P=0.6882). Respiration rates were insignificant in case of lions while these values differed only at 10$^{th}$ min (P=0.0253) interval during the reversal procedure for leopards *(Fig. 1)*.

Average pulse rates were significantly different throughout the thirty-minute interval in the case of tigers whereby P<0.0001. However, in the case of lions pulse rates were significantly different by 20$^{th}$ and 30$^{th}$ min intervals despite being similar in the beginning. While pulse rates in leopards were significant only at 10 min mark (P=0.0285) *(Fig. 2)*.

Initially at 10-min interval body temperature in tigers was nonsignificant between either of the groups, yet their values differed significantly by 20$^{th}$ (P<0.0001) and 30$^{th}$ (P<0.0001) min intervals. Lions of both groups experienced a differing body temperature at 10 and 20 min (P<0.0001), howbeit it was significant at 20 (P=0.0176) and 30 (P=0.0003) min interval intervals in case of leopards *(Fig. 3)*.

### *Recovery from Anesthesia*

Sixteen Tigers, twenty-two lions and sixteen leopards were involved in the comparative study of two anesthetic reversals i.e., tolazoline and yohimbine. All individuals studied in reversal trials were male of their respective species. Wild felids were randomly and equally divided into two groups whereby each received either of the reversal agents, post ketamine-xylazine induction. Following tolazoline (0.15 mg/kg) or yohimbine (0.15 mg/kg) administration, the onset of arousal from anesthesia was identified and elapsed time was noted by the presence of oropharyngeal reflex. Afterward, the animal started to show pedal reflex, lifted its head and moved into sternal recumbency. Finally, the time it took for the animal to stand unaided from sternal recumbency was termed to

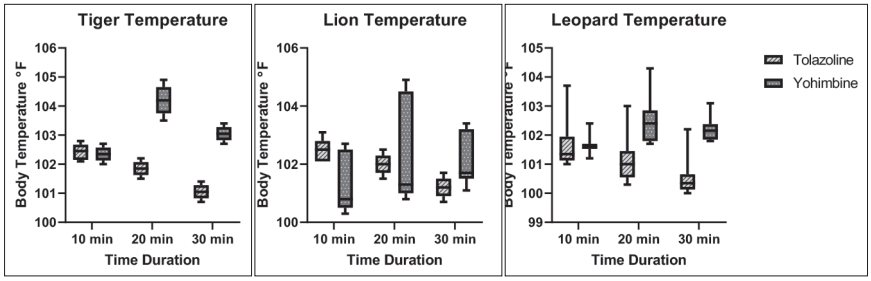


**Fig 3.** Interleaved Box and Whisker plots (GraphPad Prism Ver. 8.4.3) depicting comparative Body temperatures (°F) after tolazoline and yohimbine administration in Tiger, Lion and Leopard immobilized with ketamine-xylazine anesthesia at 10 min, 20 min, 30 min time intervals

***Table 4.*** *Comparative efficacy of tolazoline and yohimbine for anesthetic reversal in captive male tigers, lions and leopards*

| Species | Antagonist | Number of Animals | Stages of Induced Reversal | | |
|---|---|---|---|---|---|
| | | | Onset of Arousal (min) | Sternal Recumbency (min) | Standing/Recovery Time (min) |
| Tiger | Tolazoline | 8 | 3.65±0.44[a] | 7.95±0.24[a] | 12.1±0.48[a] |
| | Yohimbine | 8 | 1.04±0.16[a] | 4.5±0.31[b] | 6.3±0.58[b] |
| Lion | Tolazoline | 11 | 2.4±0.33[a] | 4.4±0.33[a] | 7.5±0.66[a] |
| | Yohimbine | 11 | 0.97±0.28[b] | 1.2±0.63[b] | 2.8±0.76[b] |
| Leopard | Tolazoline | 8 | 6.1±0.48[a] | 10.3±0.97[a] | 16.45±0.73[a] |
| | Yohimbine | 8 | 2.2±0.47[b] | 6.5±0.82[b] | 8.42±0.33[b] |

*Values are represented as mean ± SD. Different superscripts in a column within a species differ significantly whereby P<0.05*

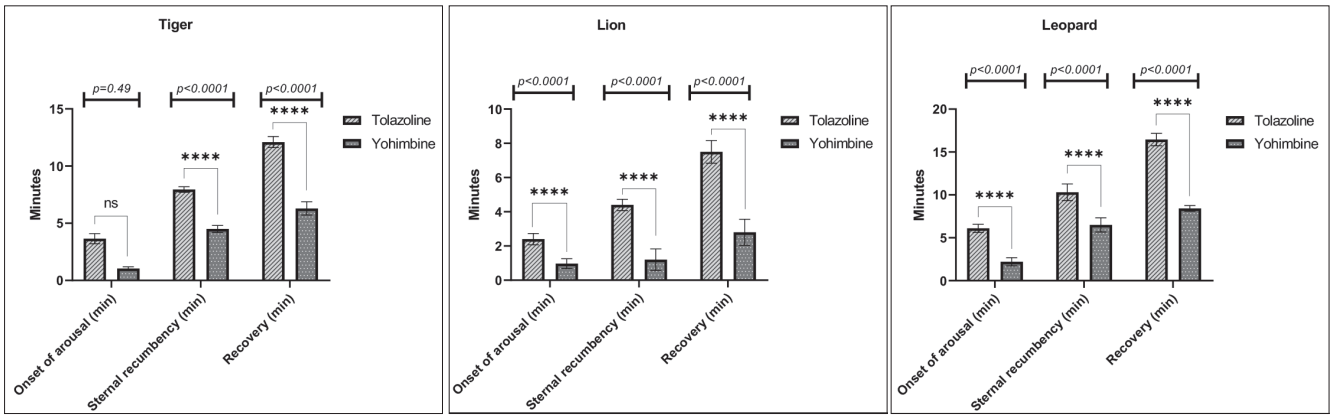


**Fig 4.** Interleaved Bar Chart (GraphPad Prism Ver. 8.4.3) depicting comparative efficacy of reversals i.e., Tolazoline and Yohimbine in tiger, lion and leopard immobilized with ketamine-xylazine anesthesia. Values are represented as mean ± SD. Significant differences (P<0.05) among groups are indicated in GP style: 0.1234 (ns), 0.0332 (*), 0.0021 (**), 0.0002 (***), <0.0001 (****)

be standing or recovery time. All these time intervals for all the species involved in the trial were collected and statistically analyzed *(Table 4)*.

The onset of arousal was significantly improved in lions (P<0.0001) and leopards (P<0.0001) while sternal recumbency and standing recovery were improved significantly for all wild felid groups administered with yohimbine. The shortest recovery period with tolazoline was observed in lions (7.5±0.66) while sternal recumbency varied between 4.4±0.33 to 10.3±0.97. However, the shortest recovery period was observed in lions administered with yohimbine (2.8±0.76). Moreover, tiger and leopards followed suite with values 6.3±0.58 and 8.42±0.33 respectively *(Fig. 4)*. In groups administered with tolazoline, although time intervals from normal unassisted recovery periods were significantly improved, however it still took much longer for tigers (12.1±0.48), lions (7.5±0.66) and leopards (16.45±0.73) in this group to recover.

### *Liver and Renal Function*

All animals involved in the trial had their blood sampled and evaluated before anesthetic administration for AST, ALT, urea and creatinine levels to establish a baseline. Groups administered with tolazoline and yohimbine had their blood sampled 12 h after the recovery to determine the deleterious effects of these drugs and compare their voracity *(Table 5)*.

In tigers, AST (P<0.0045), ALT (P<0.0072) and urea (P<0.0001)

***Table 5.*** *Comparative values of liver function test (LFT) and renal function test (RFT) in groups of captive male tigers, lions and leopards administered with Tolazoline or Yohimbine*

| Species | Values | LFT | | RFT | |
|---|---|---|---|---|---|
| | | AST (µ/L) | ALT (µ/L) | Urea (mg/dL) | Creatinine (mg/dL) |
| Tiger | Normal | 62.07±15.93[a] | 76.55±25.04[a] | 32.73±10.66[a] | 3.03±0.94[a] |
| | Tolazoline | 82.01±18.96[b] | 99.36±9.76[b] | 49.47±7.03[b] | 6.98±1.04[b] |
| | Yohimbine | 67.19±15.2[a] | 82.07±22.53[a] | 36.54±8.98[a] | 4.04±0.43[c] |
| Lion | Normal | 33.26±2.6[a] | 42.32±3.8[a] | 33.24±1.54[a] | 2.39±0.17[a] |
| | Tolazoline | 55.83±7.06[b] | 66.7±6.88[b] | 55.31±7.45[b] | 4.13±0.57[b] |
| | Yohimbine | 39.48±4.82[c] | 47.18±3.99[c] | 37.10±2.91[c] | 2.68±0.31[c] |
| Leopard | Normal | 54.66±15.99[a] | 55.46±15.07[a] | 34.8±8.76[a] | 1.23±0.57[a] |
| | Tolazoline | 87.97±18.12[b] | 84.45±11.07[b] | 56.09±8.13[b] | 2.21±0.16[b] |
| | Yohimbine | 61.23±12.31[a] | 63.26±10.02[a] | 39.63±9.49[a] | 1.44±0.43[a] |

*Values are represented as mean ± SD. Different superscripts in a column within a species differ significantly whereby P<0.05*

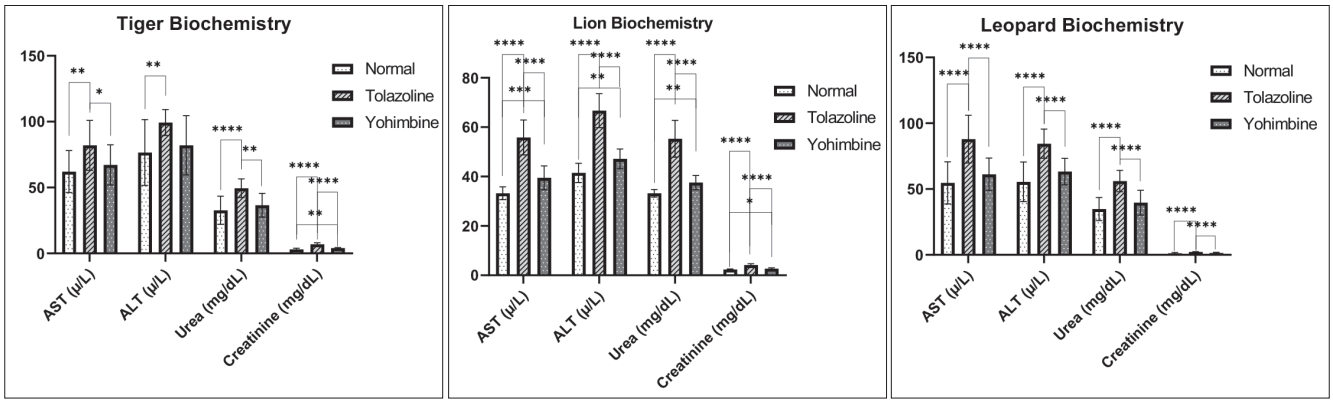


**Fig 5.** Interleaved Bar Chart (GraphPad Prism Ver. 8.4.3) depicting comparative AST, ALT, Urea and Creatinine values after Tolazoline and Yohimbine administration in tiger, lion and leopard immobilized with ketamine-xylazine anesthesia. Values are represented as mean ± SD. Significant differences (P<0.05) among groups are indicated in GP style: 0.1234 (ns), 0.0332 (*), 0.0021 (**), 0.0002 (***), <0.0001 (****)

were significantly elevated due to tolazoline from baseline values, whereas creatinine levels were significantly increased by both of the reversal agents. In lions, all the biochemical parameters were significantly different within the groups whereby values of the tolazoline group were highest in that regard. However, these values were found to be significantly greater in tolazoline when within a group post hoc analysis was performed. Similar to tigers, values in leopards when analyzed revealed that AST (P<0.0001), ALT (P<0.0001), urea (P<0.0001) and creatinine (P<0.0001) were significantly greater in animals administered with Tolazoline from normal baseline values *(Fig. 5)*.

## Discussion

The present study illustrates the efficacy of tolazoline and yohimbine for antagonizing the anesthetic effects of ketamine-xylazine combination used to immobilize lions, tigers and leopards in Pakistan. The anesthetic combinations currently being used, have shown deleterious side-effects when administered persistently. Succinyl choline hydrochloride was found to cause respiratory paralysis, while ketamine hydrochloride and phencyclidine hydrochloride had prolonged recovery periods [25]. Similar to lions, tigers exhibited unwanted side effects in response to these drugs as well. A combination of zolazepam and tiletamine has been found effective in tranquilizing wild felids, however, use in tigers has been limited as it was found to cause severe cardiopulmonary complications [26]. In certain cases, medetomidine has also been used in combination with ketamine to sedate tigers but information regarding its safe usage and effectiveness has been limited [22]. Currently, immobilization and anesthetic induction protocol of wild felids include intramuscular administration of an alpha-2 adrenergic receptor agonist, such as xylazine, medetomidine or dexmedetomidine in combination with Neuraminidase Adenine Dinucleotide (NMDA) receptor antagonist namely ketamine [11,15,19]. Ketamine is not recommended to be used as a tranquilizer or anesthetic agent separately, but only in combination with a potent sedative. It has demonstrated some side-effects such as ataxia, dysphoria, hypersalivation and hypoalgesia during induction and recovery from anesthesia. In prior studies, these side-effects have been rectified

using benzodiazepines [11]. Administration of an alpha-2 adrenergic receptor agonist in combination with ketamine allows for a reduction in the dose of anesthetic agents, thereby lowering the probability of seizures [5,27]. Cardiovascular stability also improved as lower doses of anesthetic agents were administered [28]. Consequently, in our study, animals were anesthetized with a combination of 2.6 mg/kg ketamine and 1.3 mg/kg xylazine. The efficacy of aforementioned combination is quite ubiquitous. So instead of investigating efficaciousness of these agents, previously reported dose rates were implemented. Events of overdose or contraindications were not observed amongst individuals inducted into this study. In spite of ketamine-xylazine efficacy, stimulation of alpha-2 adrenoreceptors have been found to cause increase in plasma potassium concentrations progressively. Thereby causing, hypo-insulinemia by inhibiting insulin secretion from pancreatic beta cells in feline species [9]. This hyperkalemic shift has been known to cause tachycardia initially, followed by sudden bradycardia leading to cardiac arrest and death in exotic felids [29]. Uncontrollable and unpredictable anesthetic regimens are problematic in wildlife research [30]. Consequently, this study has focused on mitigating the imperative need for reliable anesthetic antagonists in wild and captive felids. Prior studies in Bengal tiger and domestic cats have suggested the efficacy of alpha-2 antagonists in assisting the recovery of ketamine-xylazine induced anesthesia. The use of alpha-2 antagonists such as Tolazoline, yohimbine and atipamezole have been found effective in the reversal of xylazine based anesthetic regimens. Cardiac arrhythmia, tachycardia, diarrhea and vomiting and orthostatic hypotension are reported to be severe side-effects [18]. Tolazoline should be stored in cool, dark conditions as it is light-sensitive and unstable at high ambient temperatures [31]. Yohimbine, similar to tolazoline is also a potent alpha-2 adrenergic antagonist and has been employed as a reversal against ketamine-xylazine-induced sedation in wild cats [23] body weight 105-211 kg. In lions, atipamezole has also been used however the recovery period was prolonged and varied while tigers recovered within half an hour of drug administration [32,33].

In the present study, 0.15 mg/kg body weight tolazoline and yohimbine were administered intravenously in equally distributed groups of tigers, lions and leopards. The physiological parameters (temperature, pulse and respiration) were recorded immediately after the administration of reversal administration. These parameters in groups administered with tolazoline and yohimbine were consistent with previous reports [34]. Review of prior investigations into the effect of yohimbine and tolazoline antagonism on physiological norms has been corroborated by our findings whereby 10 min after its administration respiration significantly increased in all wild felids except lions [5,20,35] *(Fig. 1)*. However, pulse rates were observably lower than reported in wild felids administered with yohimbine than the ones treated with tolazoline, relating to its pronounced cardiovascular impact that has been reported previously in wolves [18,36] *(Fig. 2)*. But as time continued to elapse yohimbine, possessing greater potency, longevity and affinity in wild felids caused significant elevation in vital parameters. Improvement in respiratory rates, pulse rates and body temperature observed amongst tigers and leopards by 20$^{th}$ min of yohimbine administration as opposed to tolazoline could be justified by lower affinity of tolazoline to bind with alpha-2 adrenergic receptors [31]. Howbeit, temperature did not significantly differ in case of lions at 20 min interval, indicating physiological variation amongst different species. As most individuals continued to recover by 30$^{th}$ min, respiratory rates became non-significant amongst either of the groups. Yet, pulse rates and body temperature continued to remain significantly elevated in lions and tigers treated with yohimbine indicating greater efficacy in said species [20]. Better metabolic rate in leopards deduced from shorter recovery durations might be attributed to non-significant pulse rates at 30-min interval post reversal administration [5] *(Fig. 3)*.

When tolazoline was administered, a significant reduction in immobilization time was achieved in all cases. It allowed a return to mobility within 20-22 min of injection. Comparatively, onset of arousal was significantly improved in lions and leopards only, while sternal recumbency and standing recovery were improved significantly for all groups of species administered with yohimbine *(Fig. 4)*. The values of induced reversal stages in terms of minutes were consistent with earlier findings for antagonism with tolazoline and yohimbine [18,20]. In free-ranging animals, a quick reversal from anesthesia is imperative and the effectiveness of both reversal agents is quite consequential. Moderate return to sedation has been recorded by different authors after reversal administration [37]. But such a phenomenon was not observed in the present study, thereby indicating the doses of tolazoline and yohimbine used in our study were sufficient to antagonize the drug effects.

The adverse effects of tolazoline and yohimbine were evaluated by analyzing effects on AST, ALT, urea and creatinine. Prior studies regarding the hematological and biochemical analysis of wild felids indicated hyperglycemia and glycosuria. Both yohimbine and tolazoline have been linked to elevated levels of liver enzymes. Their adverse effects have been previously associated with hepatotoxicity and nephrotoxicity in several domestic and pet animal species [31]. But data pertaining to their impact in wild species is rather limited. Most of the prior findings have studied the impact of ketamine-xylazine combination and attributed post-anesthetic aberrations in haemato-biochemical values to xylazine rather than alpha-2 antagonist usage [24,38–40]. Nevertheless, there have been studies whereby tolazoline, yohimbine or atipamezole were employed to reverse anesthetic effects in wild felids [5,12,21,41-43].

Some of these studies have related elevated AST, ALT, urea and creatinine values with yohimbine or tolazoline usage. Similarly, we have observed pronounced effects of tolazoline on AST, ALT and urea of tiger and leopards inducted into this study as compared to yohimbine. Concomitantly, our findings in lions were vexing as both tolazoline and yohimbine significantly affected AST, ALT and urea values. Such findings in free ranging African lions have been previously reported [33]. It was suggested that higher liver enzyme values indicated liver injury while elevated serum urea and creatinine were attributed to poor clearance by kidneys [34]. Considering this fact, we can assume that just because the values were far greater when tolazoline was employed as reversal rather than yohimbine, the deleterious impact on liver and kidney was more pronounced due to tolazoline usage *(Fig. 5)*.

In the current paradigm, ketamine and xylazine combination is being used to anesthetize wild felid species in Pakistan for relocation or minor clinical procedures. This combination used in adequate dosage renders successful ataxia and effective loss of sensation. In our study we have reported that yohimbine can radically shorten the recovery period after anesthesia. The deleterious effects of yohimbine on liver and kidney function 12 h after administration were much lower than that of tolazoline.

In summary, considering the fact that yohimbine produced shorter duration for onset, sternal and standing recovery along with lower serum biochemistry values. It can therefore be concluded, that yohimbine is a far more efficacious and safer antagonist than tolazoline when used as a reversal agent in wild felids.

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## Conflict of Interest

There are no conflicts of interest in our present study.

## Author Contributions

Experimental Design was conceived by A.H. Rabbani,, Y.R. Khan and K. Hussain. Data was collected by A.H. Rabbani, A. Waheed and H. Afzal. Statistical analysis was conducted by O. Naseer, M. Shahid and A. Ali. Original draft was written by A.H. Rabbani, O. Naseer and Y.R. Khan. All authors have contributed to the revision and final proof-reading of the manuscript.

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