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Original article

EFFICACY AND SAFETY OF XYLAZINE-KETAMINE COMBINATION FOR IMMOBILISATION OF CAPTIVE AFRICAN ROCK PYTHONS (*PYTHON SEBAE*)

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Summary

Adediran, S. O., H. O. Jegede, F. M. Lawal, S. A. Amid, S. Z. Abdulkadir, A. O. Alimi, A. Aliyu & M. Bolaji, 2022. Efficacy and safety of xylazine-ketamine combination for immobilisation of captive African rock pythons (*Python sebae*). *Bulg. J. Vet. Med.,* **25**, No 2, 235–241.

The African rock python is a cosmopolitan snake in Nigeria widely kept as a zoo animal and also in recreational facilities. There is need for chemical immobilisation of this animal species for managemental, diagnostic and therapeutic procedures. A mixture of xylazine and ketamine (XK) was compared with the administration of a mixture of xylazine and normal saline solution (XS) in six captive African rock pythons in 2 trials with reference to onset and duration of anaesthesia. Changes in the heart rate (HR), respiratory rate (RR) and rectal temperature (RT) as well as selected biochemical parameters were recorded. Although there were no statistically significant ($P>0.05$) differences in HR and RR values between XK and XS treatments, significant ($P<0.05$) differences were recorded for RT. Nonetheless, the significant differences were of no clinical importance. It was therefore recommended to safely immobilise an African rock python using XK for a procedure lasting over 1 hour with minimal cardiopulmonary and plasma enzymatic effect. To the knowledge of the authors, this is the first study assessing the anaesthetic efficacy and safety in African Rock pythons.

Key words: African rock python, anaesthesia, immobilisation, ketamine, xylazine

INTRODUCTION

The field of ophidian clinical research in Nigeria has experienced rapid evolution and much attention in recent times; with works ranging from physiology (Jegede *et al*, 2017; 2020), anatomy (Jegede *et al.*, 2015), haemoparasitology (Jegede *et al.*, 2018) to anaesthesia (Abidoye *et al*., 2017).

Ketamine hydrochloride is a dissociative anaesthetic agent very popular for use in wildlife. It produces a trance-like state resulting from an electrophysiological dissociation between limbic and higher cortical system (Allen & Macias, 2005). Although its use alone in many species

has been of limited value, its effects have been improved by combination with other agents e.g. xylazine and diazepam (Green *et al*., 1981).

African rock pythons are cosmopolitan snakes in Nigeria widely kept as zoo animals and in recreational facilities, although not as prevalent as they once were due to international trade and gross persecution for meat (Jegede *et al*., 2017). With the widespread presence in captivity it is therefore imperative to perform procedures which would require anaesthesia especially with commonly available agents in this part of the world coupled with ease of administration route (e.g. intramuscularly).

This need stimulated the authors to carry out this study on the efficacy and safety of xylazine/ketamine combination as an agent for immobilisation of African rock pythons. We therefore determined the anaesthetic indices for efficacy assessment along with cardiopulmonary responses, thermoregulatory responses and plasma biochemical responses for safety assessment.

MATERIALS AND METHODS

Six (6) captive African rock pythons (*Python sebae*) were sampled in this trial. The snakes were housed in wire mesh cages and fed with chicks weekly. Water was provided *ad libitum*. All institutional and national guidelines for the care and use of animals were followed. Each snake was subjected to physical and clinical examination, morphometric measurement and weighed via a secure cotton bag placed on a scale.

Anaesthetic protocol

This experiment followed a crossover design, in which each combo was administered to each snake with a minimum 2 weeks washout period between the 2 trials, and snakes were not used during ecdysis. The pythons were manually restrained and a mixture of 2% xylazine hydrochloride solution (0.3 mg/kg) and 5% ketamine hydrochloride solution (10 mg/kg) (XK) that was administered into the dorsal epaxial muscle in the posterior third of the body, using a 23-gauge needle attached to a 2 mL syringe. A mixture of 2% xylazine hydrochloride solution (0.3 mg/kg) and 0.9% normal saline solution (ketamine equivolume) (XS) was administered on the second trial. Both experimental trials were performed in a controlled environment with ambient temperature between 27.5–27.9 °C, measured using a digital thermohygrometer (WINCOM HTC-2, China), relative humidity ranged between 50–55% .

Determination of onset and duration of anaesthesia

Parameters monitored were the presence or absence of spontaneous movement, response to tail pinch, loss of righting reflex represented by onset of anaesthesia, while recovery time was determined by observing the elicitation of righting reflex (Fig. 1).

Thermocardiopulmonary measurements

Measurement of heart rate (HR), respiratory rate (RR) and rectal temperature (RT) was done using BRAUN PM 12 Multiparameter Patient monitor. The snakes were physically restrained with a hood placed over their head, producing a calmness required for the procedure. Five probes/clips were attached on the skin with two points close to the head, one midline close to the position of the heart and two caudally at the region before the cloaca. All probes were laterally placed at

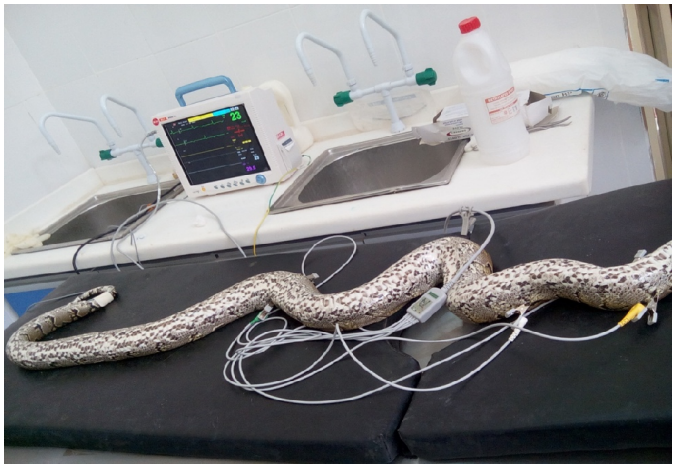

Fig. 1. ARP with lost righting reflex and being monitored.

the point where the lateral and ventral scales meet. Readings were taken multiple times for each snake.

Plasma chemistry

Blood was collected by venipuncture of the ventral coccygeal vein using a 22-gauge needle attached to a 5 mL syringe. Each snake was restrained with the tail lower than the head in order to promote blood pooling in the tail (Jegede *et al.*, 2017). Three sets of 3 mL of blood were collected from each snake (pretest, post XK and post XS (immediate post recovery) in heparinised appropriately labelled tubes (Silver Health Diagnostics, Nigeria).

The blood was spun in a centrifuge at 3000 rpm (905×g) for 15 min and plasma was decanted from the supernatant. Before biochemical processing, 50 µL of plasma was aliquoted and analysed for total protein concentration, albumin, alanine aminotransferase (ALT), aspartate transaminase (AST), uric acid and creatinine kinase using RT-9200 Rayto Chemistry Analyzer and UV spectrophotometer (Rayto Life and Analytical Sciences Co., Ltd, China).

Statistical analysis

Data were analysed using GraphPad Prism 5 (GraphPad Software Inc., La Jolla, California 92037, USA) and presented as mean ± standard deviation (SD) of six snakes. Anaesthetic indices were compared using Student *t*-test for paired data. Monitored physiological variables were analyaed using analyses of variance (ANOVA) for repeated measures and $P<0.05$ was accepted as significant.

RESULTS

Onset of anaesthesia and duration of anaesthesia with IM administration of XK were 9.3±5.1 minutes and 89.0±24.9 minutes respectively. XS produced no anaesthetic effect.

The HR ranges for XK and XS treatments were between 18.0±2.9 and 24.0±5.8, and 18.0±7.9 and 24.0±9.3 beats/min respectively (Table 1). The RR ranges for XK and XS were between 10.0±7.4 and 16.0±11.0, and 13.0±3.6 and 16.0±6.0 breaths/min respectively. The RT ranges for XK and XS were between

Table 1. Mean cardiopulmonary and thermoregulatory responses of xylazine-ketamine anaesthesia in African rock pythons. Data are expressed as mean ± SD of six snakes

	Heart rate, beats/min		Respiratory rate, breathes/min		Rectal temperature, °C	
Time interval	XK	XS	XK	XS	XK	XS
0	24±5.8	24±9.3	16±11	16±6.0	28±0.34	28±0.30
10	21±4.3	22±8.9	15±7.5	15±4.2	28±0.38	28±0.23
20	20±4.6	20±7.9	10±7.4	16±2.3	29±0.42*	29±0.26**
30	19±4.5	18±5.7	14±5.1	14±3.0	29±0.49**^{T}	29±0.43***
40	18±2.9**	19±6.5	14±7.1	14±3.5	29±0.53**^{T}	29±0.37***
50	20±5.7	18±7.9	16±4.5	14±5.0	29±0.56***^{T}	30±0.60***
60	19±5.8*	20±9.6	14±4.6	13±3.6	29±0.61***^{T}	30±0.62***

XK: xylazine-ketamine; XS: xylazine-saline; *P<0.05; **P<0.01; ***P<0.001 vs baseline (ANOVA), ^{T}P<0.05 (Student's t-test) XK vs XS.

Table 2. Mean biochemical responses of xylazine-ketamine anaesthesia in African rock pythons. Data are expressed as mean ± SD of six snakes.

Parameters	Pretest	Xylazine-ketamine	Xylazine-saline
Total protein, g/L	40±4.4	42±2.5	46±4.0
Albumin, g/L	21±2.2	23±2.0	26±1.8**
Aspartate aminotransferase, IU/L	12±4.0	14±3.9*	21±3.7**
Alanine aminotransferase, IU/L	8.2±3.3	6.3±4.1**	15±2.3*
Creatinine kinase, IU/L	8.7±2.1	5.3±3.3***	14±3.6*
Uric acid, mmol/L	0.14±0.037	0.19±0.041	0.19±0.022
Lactate dehydrogenase, IU/L	117±15	115±16***	156±15**

*P<0.05; **P<0.01; ***P<0.001 xylazine-ketamine/xylazine-saline vs pretest values.

28.0±0.4 to 29.0±0.6 °C (XK) and from 28.0±0.3 to 30.0±0.6°C (XS) (Table 1).

Plasma chemistry values for pretest, XK and XS combinations are presented in Table 2. Statistically significant increase in organ metabolites was obtained in AST, ALT, CK and LDH in the XS groups while increase in AST alone was obtained for XK (Table 2).

Although there were no relevant (P>0.05) differences between XK and XS values for HR and RR, significant (P<0.05) differences were recorded for RT between the 30th and 60th minute time intervals. Nonetheless, the significant differences were of no clinical importance.

DISCUSSION

Due to the physiologic flexibility of reptiles like poikilothermy, prolonged fasting, and swift up-regulation of the digestive system upon feed intake (Bedford & Christian, 2001; Secor & Ott, 2007), reference intervals are most times difficult to

ascertain (Campbell & Ellis, 2007). This could be a reason for the huge disparity in protein indices from the results here as compared to reference values reported earlier (Jegede *et al*., 2017), although liver enzymes were within reported ranges for the African rock python. XS combinations showed more significant increase in tissue metabolites (ALT, AST, CK and LDH) than XK that showed only mildly significant increase in AST only. Although conventional mammalian liver enzymes like ALT, AST and LDH are not considered to be organ specific, as their activity is abundant also in skeletal muscles and kidneys, they aid detecting tissue damage especially in these vital organs (Campbell, 2014). This shows XK was less deleterious than XS in this study, as XK produced negligible plasma biochemical effects.

There are various factors that affect the plasma chemistry of the ARP (Jegede *et al.*, 2017). Anaesthetic drugs have been shown to have effect on plasma chemistry of snakes where Bryant *et al.* (2012) reported a basophilic response with increased globulin concentration, although there was no statistically significant increase on total protein nor albumin in our study.

Although ketamine alone has been proven to be of clinical and anaesthetic value in snakes (Green *et al.*, 1981) and has been recommended for superficial surgeries and manipulations, the depth and duration have been questionable depending on factors like ambient temperature (Arena *et al*., 1988).

The heart rate during the period ranged between 18.0±2.9 and 24.0±5.8 beats/minute which is similar but a little lower than what was recorded by Schuszler *et al*. (2018): 23–44 beats/minute in constrictor snakes although isoflurane was added to their cocktail. Increase in temperature after the 60th minute could be due to wearing-off of anaesthetic agents and return of muscle activity which was more pronounced in the XS group since this cocktail did not have an anaesthetic effect but would definitely have had a slight sedative effect. Return or increase in muscle activity in large snakes hase been shown in literature to cause increase in cloacal temperature (Benedict & Fox, 1931).

There is currently no existing reliable system to determine the depth of anaesthesia in reptiles (Mader & Divers, 2014), so we worked mainly with the presence/absence of reaction to painful stimuli e.g. tail pinch and righting reflex. Xylazine-ketamine combination produced a safe immobilisation of 89 minutes with minimal cardiopulmonary and thermoregulatory effects which is longer than what was recorded with massive doses of ketamine alone in psammophis snake (Abidoye *et al.*, 2017). This will be much desirable as lesser doses of the drugs will be used.

Intercostal and smooth intrapulmonary muscles are affected by anaesthetics and are responsible for ventilation, in the absence of diaphragm in reptiles, apnea has been reported to occur in reptiles (Holz & Holz, 1994). Apnea was not observed in any subject therefore eliminating the need for assisted ventilation in XK use.

Only Schuszler *et al*. (2018) had used xylazine in snakes at the dose of 0.1–6 mg/kg. Ketamine however was administered at a lower limit of the recommended dose because some individuals after ketamine administration are reported to not awaken even for days (Bouts & Gasthuys, 2002) and since xylazine has been reported to enhance its effects we used the minimum possible dose even as the only

report on XK combination used 10 mg/kg in constrictor snakes (Schuszler *et al.*, 2018). Many other researchers have used higher doses up to 8 mg/kg in snakes (Bouts & Gasthuys, 2002). Sub-anaesthetic doses of ketamine produce analgesia and facilitate handling by inducing a profound immobilisation effect (Mosley, 2006), it is however unsure at this point if the effects produced are more advantageous over ketamine alone, it is definitely better than xylazine alone and also utilises less medication (dose of ketamine).

It is therefore recommended to safely immobilise African rock pythons using xylazine-ketamine for a procedure lasting over 1 hour. This, to the knowledge of the authors, is the first study of its kind in African Rock pythons.

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