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Anaesthetic Effects, Chemical Restraint, Clincophysiological and Haematobiochemical Findings after Intravenous: Detomidine/Ketamine/Midazolam Combination in Buffalo Calves

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Abstract: The present study was aimed to investigate and test the capacity of Detomidine, Ketamine and Midazolam combination (DKM) to immobilize/anaesthetize buffalo claves in addition to evaluate the changes in clinicophysiological and haematobiochemical values to produce safe and satisfactory intravenous anaesthesia for use under field conditions. Six buffalo calves, weighing 50-100 kg and aging 3-6 months were used. Each calf received in randomized order, three different doses of DKM combinations(DKM-1, DKM-2, DKM-3); 0.075 mg/kg detomidine, 5 mg/kg ketamine and 0.5 mg/kg midazolam, 0.075 mg/kg detomidine, 7.5 mg/kg ketamine and 0.5 mg/kg midazolam and 0.1mg/kg detomidine, 10 mg/kg ketamine and 0.5 mg/kg midazolam. HR, RR, RT, Na+, K+, Ca++, PO2, PCO2, HCO3, BE, Total protein, ALT, AST, Creatinine, Urea, glucose, RBCs and WBCs, PCV and Hb were recorded at 5 minutes before, then at 5, 15, 30, 45, 60, 90 minutes after anaesthesia. DKM induced a dose-dependent duration of anaesthesia, where the DKM-3 had the longest effect ($p<0.01$). Heart rate decreased significantly, while respiratory rate increased significantly and did not return to the baseline value. Intravenous injection of 0.1mg/kg detomidine, 10 mg/kg ketamine and 0.5 mg/kg midazolam (DKM-3), induces satisfactory anaesthsia and immobilization in buffalo calves although some hypoxaemia and respiratory depression was observed. Depression in heart rate was the most sever side effects. The changes in haematobiochemical findings were mild and transient. Further studies will be required to demonstrate the effectiveness of DKM-3 combination in buffalo calves in clinical settings and field conditions to quantitatively evaluate surgical anaesthesia/analgesia.

Key words: Buffalo Calves · Anaesthsia · Detomidine · Ketamine · Midazolam

INTRODUCTION

In buffalo calves, general anaesthesia and chemical immobilization is often necessary to facilitate surgical intervention or diagnostic surgical procedures and requires the administration of appropriate drugs in small volume. The ideal drug combination used in such circumstances should have a wide safety margin and short induction time. The α2-agonists are effective sedatives in buffalo calves, xylazine Hcl 2% is used to provide sedation, analgesia and immobilization including lateral recumbency and light planes of general anaesthesia [1]. However, α2-agonists cause hypoxaemia in calves [2, 3] and may induce acute pulmonary oedema in sheep [4]. Various injectable anaesthetic techniques can be used for buffalo calves under field conditions. Thiobarbiturates alone or in combination with guaiphenesin can be used for induction and maintenance of anaesthesia [5]. Ketamine is commonly used in buffalo calves alone or combined with xylazine or medetomidine [2, 6, 7]. Combination of medetomidine and ketamine produced satisfactory immobilization for umbilical surgery, although some hypoxaemia and respiratory acidosis occurred. Muscle relaxation was good and no complications were

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encountered. Tiletamine, another cyclohexanone derivative and zolazepam (a benzodiazepine) may also be used; this combination has been investigated for the use with and without xylazine [8]. Detomidine alone at the dose rate of 100 µg/kg produced only mild sedation and analgesia in buffalo calves. The addition of diazepam and ketamine to detomidine produced anaesthesia of a short duration, prolonged sedation and analgesia. The combination of detomidine-diazepam-ketamine may be used safely in buffalo calves to induce a transitory anaesthesia or chemical immobilization for clinical examination [9]. The pharmacological actions of midazolam are identical to those of other benzodiazepines including induction of sleep, sedation, anxiolysis and amnesia. Midazolam differs from other agents by virtue of its more rapid onset of clinical effects and shorter duration of action. Midazolam belongs to a newer class of benzodiazepines called imidazobenzodiazepines [10]. However, no many studies have been performed in buffalo calves to assess the efficacy of such combinations and their potential side effects, so the objective of this randomized study was to investigate and test the capacity of Detomidine, Ketamine and Midazolam combination (DKM) to immobilize/anaesthetize buffalo claves in addition to evaluate the changes in clinicophysiological and haematobiochemical values to produce safe and satisfactory intravenous anaesthesia for use under field conditions.

MATERIALS AND METHODS

Buffalo Calves: The present study was approved by the Committee of Animal Welfare and Ethics at Alexandria University. Six healthy buffalo calves, weighing 50-100 kg and aging 3-6 months were used. The buffalo calves were in a good health determined by the physical examination. All buffalo calves were housed in stalls in the department of surgery, at the Faculty of Veterinary Medicine, Alexandria University.

Study Design: Each calf received, in random order, three different doses of the following DKM combination; 0.075 mg/kg body weight detomidine, 5 mg/kg body weight ketamine and 0.5 mg/kg body weight midazolam (DKM-1), 0.075 mg/kg body weight detomidine, 7.5 mg/kg body weight ketamine and 0.5 mg/kg body weight midazolam (DKM-2) and 0.1mg/kg body weight detomidine, 10 mg/kg body weight ketamine and 0.5 mg/kg body weight midazolam (DKM-3). The appropriate doses of detomidine (Domosedan; Orion Corporation Farmos, Finland), Ketamine HCl (Alfamyne; Egevet, Turkey) and Midazolam (Demizolam; Dem, Turkey) were mixed and used to induce immobilization and anaesthesia in the six buffalo calves. The intravenous treatment was applied to each buffalo calf with a minimum of one-week interval. The day before study commencement, the buffalo calves were clinically examined and weighed. Food, but not water, was withheld on the day of the study. The site of the injection was aseptically prepared, clipped and scrubbed with povidine iodine solution. The detomidine/ketamine/midazolam combinations were administered by intravenous injection through jugular vein. Onset of first signs of sedation, onset of sternal recumbence, onset of lateral recumbence, time to first movement, duration of anaesthesia and duration of recumbence were recorded and analyzed. Pain was assessed by applying a standard noxious stimulus to the buffalo calves through the skin and deep muscle pinprick of perineum with a 20 gauge, 3-cm-long needle for evaluation of analgesia. Two response scales were developed to quantify the responses of buffalo calves. The response to the pinprick in the skin and the perineal region was scored as: excellent (1, no response) or poor (0, very responsive with severe muscle fasciculation). All buffalo calves were left to stand quietly for approximately 1 hour in the restraining device before base-line data recording began. Heat rate (HR, bpm), respiratory rate (RR, breaths/ min), rectal temperature (RT), sodium (Na+), potassium (K+), ionized calcium (Ca++), arterial pH (pH), Partial pressure of arterial oxygen (PO2), partial pressure of carbon dioxide in arterial gas (PCO2), bicarbonate concentration (HCO3) and base excess (BE) were measured at 5 minutes before injection (baseline values), then at 5, 15, 30, 45, 60 and 90 minutes after injection of the detomidine/ketamine/midazolam. Respiratory rate was determined by the direct observation. Arterial blood samples were collected into heparinized syringes for the measurement of electrolytes and blood gas values. Air was removed from the blood gas syringe and the sample was immediately analyzed using an automated blood gas analyzer (Model ITC Irma Trupoint, Blood analysis System, ITC, USA). Analysis of sodium (Na+), potassium (K+) and ionized calcium (Ca++) were performed using commercially available kits (Biomedical Systems, Spain). Venous blood samples were collected at the previous intervals for both hematological and biochemical analysis of total protein ALT and AST, Creatinine, Urea and Serum glucose. To determine the counts of RBCs and WBCs and values of PCV% and Hb concentration, the blood was collected into heparinized tubes at 5 minutes before injection (baseline values), then at 5, 15, 30, 45, 60 and 90

minutes after induction of anaesthesia with groups of DKM combination and analyzed by a automatic cell counter. The buffalo calves were observed continuously during the recovery period until they were standing.

Statistical Analysis: The data were reported as mean ± SD. Analysis of variance (ANOVA) was used for repeated measures to analyze the continuous numerical data (Prism version 4.0; Graph Pad Software, Inc., San Diego, CA, USA). Dunnet's and Tukey's post-tests were used to detect the differences from baseline value and among time points, respectively. The data for Heat rate, respiratory rate, rectal temperature, sodium (Na+), potassium (K+), ionized calcium (Ca++), arterial pH, PO2, PCO2, HCO3, BE, Total protein, ALT, AST, Creatinine, Urea, Serum glucose, counts of RBCS and WBCS, PCV% and Hb concentration were grouped, analyzed and summarized as means ± SD. Similarly, the non-repeated data as onset of first signs of sedation, onset of sternal recumbence, onset of lateral recumbence, time to first movement, duration of anaesthesia and duration of recumbency were analyzed using a one-way analysis of variance. In each analysis, the differences were considered a significant if $p< 0.01$ [11].

RESULTS

Anaesthetic Status: The buffalo calves treated with detomidine/ketamine/midazolam combinations showed the first signs of sedation including sunken of the head and reduced awareness at 0.93±0.03, 0.87±0.07 and 0.70±0.02 minutes after the administration of DKM-1, DKM-2 and DKM-3, respectively. The buffalo calves lay down and the onset of sternal recumbency was observed at 1.7±0.70, 1.5±0.50 and 1.1±0.10 minutes after injection of the previous combinations. The calves were deeply sedated in lateral recumbency after 2.1±0.01, 2.1±0.01 and1.7±0.02 minutes after the administration of DKM-1, DKM-2 and DKM-3, respectively (Table 1). As used in this study, detomidine/ketamine/midazolam injection induced a dose-dependent duration of anaesthesia, time to first movement and duration of recumbency, where the DKM-3 had the longest effect ($p<0.01$) and the DKM-1 had the shortest effect. The durations of anaesthesia and recumbency for the different doses of DKM combination are illustrated in (Table 1 and Fig. 1). Absence of the response to the pinprick stimulus in the skin and deep muscle of perineum was observed in all treated buffalo calves, although the duration of this effect was short-lived with the DKM-1 and longer with both DKM-2 and DKM-3 combinations.

Clinicophysiological Effects: All three doses induced significant, dose-dependent changes in cardio-respiratory variables (Table 2 and Fig. 2). Heart rate decreased significantly in all buffalo calves treated with all doses of detomidine/ketamine/midazolam combinations. There was a statistically significant decrease ($p<0.01$) in the mean heart rate within 5 minutes after the injection, then it began to increase gradually at 60 and 90 minutes to be around the baseline values. Respiratory rate increased significantly with all doses of detomidine/ketamine/ midazolam combinations and never return to the baseline values at the end of the experiment. The rectal temperature showed a non-significant decrease during the anaesthesia except at 60 and 90 minutes after injection of DKM-3 it decreased significantly ($p<0.01$) and did not return to the baseline value at the end of the experiment (Table 2 and Fig. 2). The serum sodium (Na+), potassium (K+) and ionized calcium (Ca++), decreased significantly ($p<0.01$) with the time during the periods of anaesthesia in all buffalo calves treated with detomidine/ketamine/ midazolam. The ionized calcium returned to the baseline

Table 1: Anaesthetic status of buffalo calves after intravenous administrations of: 0.075mg/kg detomidine, 5mg/kg ketamine and 0.5mg/kg midazolam (DKM-1), 0.075mg/kg detomidine, 7.5mg/kg ketamine and 0.5mg/kg midazolam (DKM-2) and 0.1mg/kg detomidine, 10 mg/kg ketamine and 0.5mg/kg midazolam (DKM-3). Values are Mean ± SD

	Anaesthetic status of buffalo calves					
Anaesthetic drugs combinations	Onset of first signs of sedation (min) Mean ± SD	Onset of sternal recumbancy (min) Mean ± SD	Onset of lateral recumbancy (min) Mean ± SD	Time to first movement (min) Mean ± SD	Duration of anaesthesia (min) Mean ± SD	Duration of recumbency (min) Mean ± SD
DKM-1	0.93±0.03 A	1.7±0.70 A	2.1±0.01 A	51±5.43 C	45±4.63 C	82±8.12 C
DKM-2	0.87±0.07 A	1.5±0.50 AB	1.9±0.02 AB	56±4.22 B	50±5.52 B	103±9.32B
DKM-3	0.70±0.02 A	1.1±0.10 B	1.7±0.02 B	66±5.56 A	60±4.66 A	115±11.13A

No of Buffalo calves=6

Means within the same column of different letters are significantly different at ($p< 0.01$).

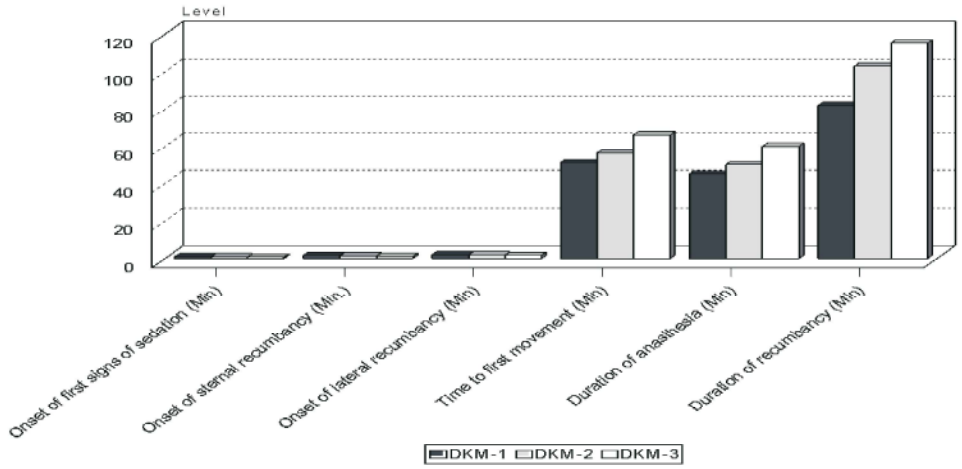

Fig. 1: Showing anaesthetic status of buffalo calves after intravenous administration of: 0.075mg/kg detomidine, 5mg/kg ketamine and 0.5mg/kg midazolam (DKM-1); 0.075mg/kg detomidine, 7.5mg/kg ketamine and 0.5mg/kg midazolam (DKM-2), and 0.1mg/kg detomidine, 10 mg/kg ketamine and 0.5mg/kg midazolam (DKM-3).

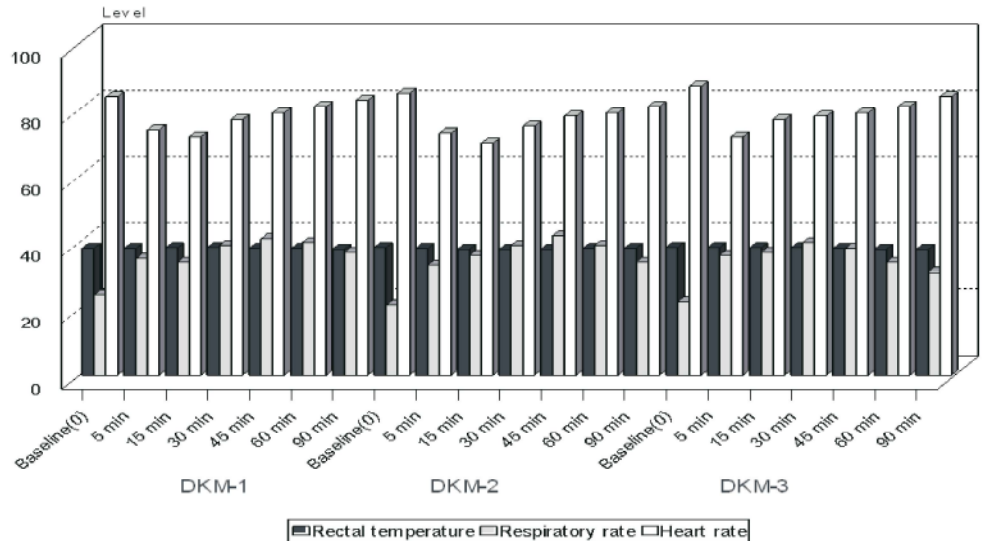

Fig. 2: Showing Rectal temperature, Respiratory rate, Heart rate in buffalo calves before and after intravenous administration of: 0.075mg/kg detomidine, 5mg/kg ketamine and 0.5mg/kg midazolam (DKM-1); 0.075mg/kg detomidine, 7.5mg/kg ketamine and 0.5mg/kg midazolam (DKM-2) and 0.1mg/kg detomidine, 10mg/kg ketamine and 0.5mg/kg midazolam (DKM-3

values in buffalo calves treated with intravenous injection of both DKM-2 and DKM-3 combinations (Table 2). The PO2 significantly decreased and the PCO2 significantly increased between 15 and 45 minutes at all doses of the detomidine/ketamine/midazolam combination ($P<0.01$) and never return to the baseline values (Table 3). The pH was decreased significantly at all observed time points with each of the three doses and remained low until 90 minutes after induction of anaesthesia. All buffalo calves were acidosis. The lowest individual arterial pH measured in this study was observed at 45 minutes in all doses of detomidine/ketamine/midazolam. The significant reduction of the bicarbonate concentration was also observed between 5 and 45 minutes, after that it gradually increased to be around the baseline values at the end of the experiment. The base extract values varied between significant decrease to significant increase ($P<0.01$) at all doses of detomidine/ketamine/midazolam (Table 3).

Table 2: RT, RR, HR, Sodium, Potassium and Calcium ions in buffalo calves before and after intravenous administrations of: 0.075mg/kg detomidine, 5mg/kg ketamine and 0.5mg/kg midazolam (DKM-1), 0.075mg/kg detomidine, 7.5mg/kg Ketamine and 0.5mg/kg midazolam (DKM-2) and 0.1mg/kg detomidine, 10mg/kg ketamine and 0.5mg/kg midazolam (DKM-3). Values are Mean ± SD

Anaesthetic drugs combinations	Time/min	RT (°C) Mean ± SD	RR(breaths/min) Mean ± SD	HR (bpm) Mean ± SD	Sodium (Na+) (mmol/L) Mean ± SD	Potassium (K+) (mmol/L) Mean ± SD	Calcium (Ca+) (mmol/L) Mean ± SD
DKM-1	Baseline (0)	38.2±3.2a	24±2.4h	84±4.9a	136±6.4b	4.6±1.4a	1.3±0.3a
	5 min	38.1±3.2a	35±2.3e	74±4.8d	134±4.3c	4.3±1.4a	1.3±0.3a
	15 min	38.3±3.2a	34±2.3e	72±3.8d	131±3.3c	3.9±1.4b	1.2±0.2b
	30 min	38.4±3.1a	39±2.3b	77±3.8c	132±3.2c	4.0±1.3b	1.2±0.2b
	45 min	38.1±3.2a	41±3.2a	79±3.8c	133±3.3c	3.9±1.4b	1.2±0.2b
	60 min	38±3.2a	40±3.2a	81±2.9b	134±4.3b	4.1±1.1a	1.2±0.3b
	90 min	37.9±3.7b	37±2.4c	83±3.9a	135±3.3bc	4.4±1.4a	1.3±0.3a
DKM-2	Baseline (0)	38.4±3.1a	21±2.4h	85±5.9a	138±3.4a	4.7±1.5a	1.3±0.3a
	5 min	38.2±3.2a	33±3.3f	73±3.8d	137±3.4ab	4.5±1.4a	1.3±0.3a
	15 min	37.9±3.4b	36±3.3d	70±2.8d	135±3.3bc	4.2±1.2a	1.2±0.2b
	30 min	37.8±3.4b	39±2.4b	75±2.8cd	134±3.3bc	3.9±1.2b	1.3±0.3b
	45 min	37.9±3.3b	42±2.4a	78±3.8c	135±3.3bc	3.8±1.4b	1.2±0.2b
	60 min	38.1±3.4a	39±2.3b	79±2.8c	136±3.4b	4.0±1.9b	1.2±0.3b
	90 min	38.2±3.3a	34±2.4f	81±2.9b	137±3.4b	4.2±1.4a	1.3±0.2b
DKM-3	Baseline (0)	38.5±3.4a	22±2.2i	87±2.8a	139±4.4a	4.4±1.4a	1.3±0.3a
	5 min	38.4±3.3a	36±2.4c	72±2.8 d	137±4.4b	4.2±1.4a	1.3±0.3a
	15 min	38.3±3.4a	37±2.4c	77±2.8c	132±3.2c	4.0±1.4b	1.3±0.3b
	30 min	38.3±3.1a	40±2.4a	78±2.4c	134±3.4bc	3.9±1.4b	1.3±0.3b
	45 min	38±3.2a	38±2.3b	79±2.8c	135±5.3bc	4.0±1.4b	1.2±0.2b
	60 min	37.9±3.2b	34±2.3e	81±2.9b	136±3.4b	4.1±1.4b	1.3±0.3b
	90 min	37.8±3.4b	31±2.3g	84±2.8a	138±3.4a	4.2±1.4a	1.3±0.3a

No of Buffalo calves=6

Means within the same column of different letters are significantly different at ($p< 0.01$).

Table 3: Mean ± SD of Arterial blood gas, pH, Base excess and Bicarbonate concentration in six buffalo calves before and after intravenous administration of: 0.075mg/kg detomidine, 5mg/kg ketamine and 0.5mg/kg midazolam (DKM-1), 0.075 mg/kg detomidine, 7.5mg/kg ketamine and 0.5mg/kg midazolam (DKM-2) and 0.1mg/kg detomidine, 10 mg/kg ketamine and 0.5mg/kg midazolam (DKM-3). CO2 partial pressure. Values are Mean ± SD

Anaesthetic drugs combinations	Time/min	pH (units) Mean ± SD	PCO2 (mmHg) Mean ± SD	PO2 (mmHg) Mean ± SD	HCO3 (mmol/L) Mean ± SD	BE (mmol/L) Mean ± SD
DKM-1	Baseline (0)	7.45 ± 0.04a	48±4.7a	99.6 ± 8.8b	97.6 ± 1.7a	5.4 ± 1.7a
	5 min	7.41 ± 0.03a	55±3.3 b	89.2 ± 7.9c	80.2 ± 10.4b	5.1 ± 1.5a
	15 min	7.39 ± 0.02b	58±2.3 b	77.8 ± 16.8d	77.2 ± 9.4c	5.3 ± 1.4a
	30 min	7.37 ± 0.02b	60.4 ± 4.3c	54.0 ± 13.3d	91.2 ± 2.1d	5.9 ± 1.3b
	45 min	7.33 ± 0.02c	61±4.3 c	75.5 ± 10.8d	94.3 ± 1.5d	6.4 ± 1.5c
	60 min	7.36 ± 0.01bc	56.3 ± 4.8b	90.4 ± 8.3c	95.4 ± 1.4d	7.1 ± 1.1c
	90 min	7.39 ± 0.02 b	53.6 ± 5.3b	95.2 ± 8.5c	96.1 ± 1.3a	7.6 ± 1.2c
DKM-2	Baseline (0)	7.42 ± 0.03 a	47.5 ± 4.5 a	98.8 ± 10.0 b	97.1 ± 1.9a	4.9 ± 2.0a
	5 min	7.40 ± 0.04a	58±2.3 b	90.6 ± 6.3c	87.3 ± 7.6b	4.1 ± 2.6b
	15 min	7.39 ± 0.03 b	61±0.24 b	46.8 ± 5.2d	71.5 ± 7.9c	3.8 ± 2.9c
	30 min	7.36 ± 0.03b	63.0 ± 4.2c	7 5.0 ± 12.3d	80.7 ± 10.1d	4.7 ± 2.2c
	45 min	7.32 ± 0.05c	64±3.7c	77.8 ± 16.8d	91.5 ± 2.9d	5.1 ± 2.2c
	60 min	7.35 ± 0.03 c	60±4.1b	89.4 ± 7.8c	95.8 ± 3.6d	6.3 ± 1.8d
	90 min	7.38 ± 0.03 b	53.8 ± 3.4c	86.8 ± 6.8d	95.3 ± 3.6d	6.4 ± 2.7d
DKM-3	Baseline (0)	7.43 ± 0.04a	47.7±4.6a	98.6 ± 21.3 b	96.9 ± 2.1a	5.3 ± 1.6a
	5 min	7.38 ± 0.03b	54.5±3.3 b	93±5.1 c	78.5± 9.4c	4.9 ± 2.1a
	15 min	7.37 ± 0.03b	55±2.7 b	78.5±3.7 d	68.4 ± 7.5c	5.3 ± 1.8a
	30 min	7.35 ± 0.03b	58±2.3 b	72.6±6.3 d	90.1± 3.1b	5.6 ± 1.7b
	45 min	7.33 ± 0.02c	64±3.1c	74.4 ± 7.8 d	94.1 ± 2.8a	6.1 ± 1.3d
	60 min	7.36 ± 0.02c	66.6 ± 4.4c	77.8 ± 16.8 d	94.3 ± 2.5a	6.3 ± 1.5d
	90 min	7.38 ± 0.03b	62.6 ± 3.8c	85.8 ± 23.7c	94.6 ± 3.2a	6.9± 0.9d

No of Buffalo calves=6

Means within the same column of different letters are significantly different at ($p< 0.01$).

Table 4: Mean ± SD of ALT, AST, TP, Urea, Creatinine and Glucose in buffalo calves before and after intravenous administration of: 0.075 mg/kg detomidine, 5mg/kg ketamine and 0.5mg/kg midazolam (DKM-1), 0.075mg/kg detomidine, 7.5mg/kg ketamine and 0.5mg/kg midazolam (DKM-2) and 0.1mg/kg detomidine, 10 mg/kg ketamine and 0.5 mg/kg midazolam (DKM-3). Values are Mean ± SD

Anaesthetic drugs combinations	Time/min	ALT(U/L) Mean ± SD	AST(U/L) Mean ± SD	TP(g/dl) Mean ± SD	Urea(mmol/L) Mean ± SD	Creatinine(µmol/L) Mean ± SD	Glucose(mmol/L) Mean ± SD
KM-1	Baseline (0)	84±4.9b	24±2.4d	38.2±2.3a	26.3±2.4c	1.4±0.42b	74.3±3.8c
	5 min	74±4.8e	35±3.5c	38.1±3.8a	32.2±3.2b	1.5±0.55a	90.5±5.3a
	15 min	72±2.8e	34±3.3c	38.3±3.4a	38.9±3.3a	1.6±0.57a	89.4±4.7a
	30 min	77±4.8d	39±3.4b	38.4±3.8a	34±3.4b	1.7±0.72a	83.8±8.4b
	45 min	79±5.8d	41±3.4b	38.1±3.8a	36.1±3.4ab	1.6±0.66a	81.7±7.9b
	60 min	81±4.9c	40±3.4b	38±3.4a	27.5±3.2c	1.6±0.55a	79.3±3.8b
	90 min	83±4.9b	37±3.8c	37.9±3.4b	26.3±2.6c	1.5±0.44b	76.3±3.7c
DKM-2	Baseline (0)	85±4.9b	21±2.2e	38.4±3.8a	24.8±2.1c	1.3±0.3b	68.5±5.7c
	5 min	73±4.7e	33±3.3c	38.2±3.4a	33.2±2.3b	1.4±0.4b	83.5±3.9b
	15 min	70±4.8e	36±3.7c	37.9±3.4b	38.9±2.4a	1.4±0.4b	79.7±7.8b
	30 min	75±5.8d	39±3.4b	37.8±3.36b	35±2.3b	1.5±0.5a	81.9±2.4
	45 min	78±4.8d	42±2.4a	37.9±3.4b	33.1±2.3b	1.4±0.4b	82.8±4.7b
	60 min	79±4.8d	39±3.4b	38.1±3.4a	27.5±2.3c	1.4±0.4b	77.3±3.8c
	90 min	81±4.8c	34±3.3c	38.2±3.4a	25.1±2.3c	1.3±0.3b	71.1±2.7d
DKM-3	Baseline (0)	87±4.8a	22±2.2e	38.5±3.4a	24.6±2.5c	1.2±0.2b	64.3±3.4e
	5 min	72±2.8e	37±3.4c	38.4±3.4a	33.2±2.3c	1.4±0.4b	80.5±5.8b
	15 min	77±4.8d	36±3.4c	38.3±3.4a	38.9±2.4a	1.5±0.4b	79.4±4.8b
	30 min	78±4.8d	43±3.4a	38.3±3.3a	35±3.4bc	1.6±0.4a	81.8±4.7b
	45 min	79±4.7cd	45±3.5a	38±3.4a	33.1±2.4b	1.7±0.4a	83.7±3.8b
	60 min	80±4.8c	44±4.4a	37.9±3.4b	27.5±2.4c	1.6±0.4a	71.3±4.8d
	90 min	82±4.8c	40±4.4b	37.8±3.4b	25.1±2.4c	1.3±0.3b	61.3±6.3f

No of Buffalo calves=6

Means within the same column of different letters are significantly different at ($p< 0.01$).

Table 5: Hb, PCV%, RBCs counts and WBCs counts in buffalo calves before and after intravenous administration of: 0.075mg/kg detomidine, 5mg/kg ketamine and 0.5mg/kg midazolam (DKM-1), 0.075 mg/kg detomidine, 7.5mg/kg ketamine and 0.5mg/kg midazolam (DKM-2) and 0.1mg/kg detomidine, 10 mg/kg ketamine and 0.5mg/kg midazolam (DKM-3). Values are Mean ± SD

Anaesthetic drugs combinations	Time/min	Hb (g/dl) Mean ± SD	PCV % Mean ± SD	RBCs($x10^6$ / mL) Mean ± SD	WBCs ($x10^3$/ mL) Mean ± SD
DKM-1	Baseline (0)	11.3±2.9a	30.2±4.4b	7.1±1.3a	17.4±2.2a
	5 min	10.9±2.1b	29.5±4.9bc	6.7±1.3b	15.3±2.2b
	15 min	10.6±2.1b	28.6±4.3c	6.3±1.3b	14.2±2.2b
	30 min	10.0±2.9c	27.2±4.3c	5.9±1.1c	13.6±2.2c
	45 min	10.1±2.2b	26.8±4.9d	6.0±1.2c	12.8±2.2c
	60 min	10.9±2.9b	28. 7±4.9c	6.4±1.2b	17.6±2.2a
	90 min	11.1±2.1a	29.6±4.9bc	7.0±1.2b	18.6±2.2a
DKM-2	Baseline (0)	11.0±2.9b	29.8±4.4bc	6.8±1.2b	17.1±2.2a
	5 min	10.9±2.9b	28.5±4.5c	6.3±1.2c	14.3±2.1b
	15 min	9.9±3.0c	27.6±4.5c	6.2±1.2c	12.2±2.1c
	30 min	9.8±2.9c	26.8±4.3d	5.9±1.1c	11.1±2.1c
	45 min	9.9±3.0c	27.4±4.8c	6.0±1.2c	11.5±2.1c
	60 min	10.3±2.3b	38.5±5.3a	6.2±1.2c	17. 9±2.2a
	90 min	10.8±2.1b	29.3±4.5bc	6.7±1.2b	18.7±2.2a
DKM-3	Baseline (0)	10.9±2.9b	28.8±4.5b	6.9±1.2b	16.6±2.2ab
	5 min	10.8±2.9b	27.5±5.3c	6.3±1.2b	13.3±2.1c
	15 min	9.9±2.9c	26.6±3.7d	5.8±1.1c	12.2±2.1c
	30 min	9.8±2.7c	26.2±3.7d	5.7±1.2c	12.6±2.2c
	45 min	9.8±2.8c	26.4±4.3d	5.8±1.2c	11.8±2.1c
	60 min	11.2±2.1a	30.5±5.3a	6.5±1.2b	18.6±2.2a
	90 min	11.4±2.1a	30.7±5.3a	6.9±1.2b	18.9±2.2a

No of Buffalo calves=6

Means within the same column of different letters are significantly different at ($p< 0.01$).

Haematobiochemical Findings: The ALT activity decreased significantly ($p<0.01$) with the time during the anaesthesia in all groups of detomidine/ketamine/ midazolam and began to return to the baseline values at 90 minutes, while The AST activity increased significantly in all periods of observation during the anaesthesia and did not return to the baseline value. The values of total protein varied between non-significant to significant decrease during the periods of anaesthesia. In DKM-3 treated buffalo calves, the total protein value reached to 37.9±3.4 and 37.8±3.4 g/dl at 60 and 90 minutes, respectively, (Table 4). The serum values of urea concentrations showed a significant increase ($p<0.01$) in all groups at 45 minutes after injection, then it decreased significantly until returned to the baseline values. The creatinine values varied between non-significant to significant increase during the observation period of anaesthesia after intravenous injection of all groups of detomidine/ketamine/midazolam combinations in buffalo calves. The serum glucose concentration increased significantly ($p<0.01$) during the anaesthesia in all buffalo calves treated with detomidine/ketamine/midazolam, after that it decreased significantly until returned to the baseline values (Table 4). Hemoglobin concentrations decreased significantly ($p<0.01$) in all groups at 45 minutes after injection, then it increased significantly to reach 11.1±2.1, 10.8±2.1 and 11.4±2.1 g/dl at the end of the experiment in buffalo calves treated with DKM-1, DKM-2 and DKM-3, respectively. The PCV% decreased significantly for a short time in all buffalo calves after the injection of the compounds. At the end of the experiment, it returned to the baseline value in both DKM-1 and DKM-2 combinations but in buffalo calves treated with intravenous injection of DKM-3, the PCV% showed a significant increase until reached to 30.5±5.3 and 30.7±5.3 at 60 and 90 minutes, respectively (Table 5). The RBCs counts decreased significantly ($p<0.01$) in buffalo calves treated with intravenous injection of DKM-1, DKM-2 and DKM-3 combinations, after that it increased significantly until returned to the baseline values. All buffalo calves treated with detomidine/ketamine/midazolam combinations exhibited a significant decrease in WBCs counts at 45 minutes after injections in all treatment groups, after that it showed a non-significant increase at 60 and 90 minutes after injection until returned to the baseline values (Table 5).

DISCUSSION

This study found that intravenous administration of detomidine/ketamine/midazolam combination induces dose-dependent anaesthesia, where the highest dose DKM-3 had the longest effect, which induces a period of anaesthesia lasted for 60±4.66 minutes and the lowest dose DKM-1 had the shortest effect, which induces a period of anaesthesia lasted for 45±4.63 minutes in buffalo calves ($p<0.01$). Immobilization lasted for 66±5.56 minutes with the highest dose used, a time-scale suitable for carrying out the most clinical procedures. However, our findings also indicate that this drug combination is not without server side-effects in that mild depression in heart rate but not induces respiratory depression. Ketamine-based anaesthesia is commonly used in domestic and wild ruminants and when combined with sedatives such as α2-adrenoceptor agonists and/or benzodiazepines [6, 7, 9, 12-14], the anaesthetic effects are comparable to those achieved with the drug combination used in this study. A combination of Detomidine (0.1mg/kg), Ketamine (10 mg/kg) and Midazolam (0.5 mg/kg) has been used to immobilize/anaesthetise buffalo calves. In this species, the effect of the drug combination has a relatively prolonged onset and recovery time and creates adequate anaesthetic depth and muscle relaxation to facilitate minor surgical procedures [14, 15]. In the current study, the analgesia was assessed using pin-prick test through the skin and deep muscle of perineum, rather than true 'surgical' stimuli [16]. The doses used are likely to be appropriate for use for the most procedures in buffalo calves under field conditions. In such circumstances additional local analgesia can be provided if required. The side-effects of DKM combination have been found with other similar drug combinations [7, 12-14, 17, 18]. In this study heart rate decreased significantly in buffalo calves treated with detomidine/ketamine/midazolam. Bradycardia was found at all doses, probably due to the effect of detomidine [19, 20], although this would be counter-acted to an extent by ketamine [9, 20, 21]. Respiratory rate increased significantly in all groups of detomidine/ketamine/ midazolam combinations. Rectal temperature decreased in this study in all groups of DKM combinations during the periods of anaesthesia except at 60 and 90 minutes in DKM-3. The decrease in RT was recorded following systemic administration of α2-adrenoceptor agonists [22], which was attributed to the depression of the hypothalamic thermoregulatory centre, reduced basal metabolic rate and muscle activity [23, 24]. In the present study the changes in serum electrolytes of Na+, K+ and Ca++ were mild, transient and all values returned to the baseline values at the end of experiment in groups of combinations [15]. In our study after administration of all doses of detomidine/ketamine/midazolam to buffalo calves PCO2 increased and heart rate, PO2 and pH decreased.

The same results have been reported in the calves after administration of medetomidine and ketamine [12]. Hypoxaemia is common in ruminants anaesthetised with α2-based drug combinations [18], although ketamine may also account for this effect in an additive fashion [12]. Other factors that may contribute to hypoxaemia include the positioning of the calves in lateral recumbency which is associated with ruminal tympany. Sternal recumbency reduces the compressive effect of the abdominal viscera on the lungs and so was the position of choice for our study. Also, calves may sometimes be positioned in an 'anti-Trendelenburg' position with the surgical table tilted cranially [25]. However, given that sternal recumbency was associated with hypoxaemia in our study, the administration of oxygen under this anaesthetic is advisable [7]. Although not used in this study, the Detomidine antagonist atipamezole could be used to reverse respiratory depression [26]. The ALT activity decreased significantly ($p<0.01$) with the time during the anaesthesia and returned to the baseline values [15], the possibility of pathological changes in the liver could therefore, be ruled out without dangerous adverse effects on hepatic function. It corroborates with the findings of [27], after detomidine administration in cattle and sheep. The values of total protein varied between non-significant to significant decrease among buffalo calves treated with DKM. A significant increase in serum urea ($p<0.01$) was recorded in all groups at 45 minutes, then it decreased significantly until returned to the baseline values. This might be attributed to a temporary inhibitory effect of the drug on the renal blood flow [27]. The serum creatinine values showed non-significant to significant increase in all groups in buffalo calves [15]. The increase in serum creatinine might be attributed to the temporary inhibitory effect of these drugs on the renal blood flow, which in turn might have caused a rise in serum creatinine values. However, it is difficult to attribute this to the possible renal damage, because all the reported values were within normal physiological limits [28]. The values for serum glucose concentration increased significantly ($p<0.01$) in all buffalo calves treated with detomidine/ketamine/ midazolam, then it returned to the baseline values [15]. Hyperglycemic effects of α2-adrenoceptor agonists are well known. The hyperglycemic effect might be to the result of α2-adrenergic receptor inhibition of insulin release by the stimulation of α2-adrenoreceptors in the pancreatic *â* cells [29] and to an increased glucose production in the liver (Hsu and Hummel 1981) [30]. In the current study hemoglobin concentrations decreased significantly ($p<0.01$) in all groups at 45 minutes, then it increased until returned to the baseline value. The PCV% decreased significantly for a short time in all buffalo calves and it returned to the baseline value in both DKM-1 and DKM-2, while in buffalo calves treated with DKM-3, the PCV% showed a significant increase until returned to be around the baseline values [15]. The decrease in PCV% and Hb concentration during the periods of anaesthesia might be attributed to the shifting of fluid from extra vascular compartment to intravascular compartment in order to maintain normal cardiac output in the animals [31]. The counts of RBCs decreased significantly ($p<0.01$) in buffalo calves treated with intravenous injection of DKM combination, then it increased significantly until returned to the baseline values. The values for WBCs counts decreased significantly at 45 minutes, after that it increased until returned to the baseline values [15]. The effectiveness of α_2 agonists on RBCs and WBCs counts was fluctuated and within the physiological limit. Pooling of circulating blood cells in the spleen and other reservoirs secondary to decreased sympathetic activity could be the reason for a decrease in Hb concentration, PCV% and WBC counts [9, 32].

Intravenous injection of 0.1mg/kg detomidine, 10 mg/kg ketamine and 0.5 mg/kg midazolam (DKM-3), induces satisfactory anaesthsia and immobilization in buffalo calves although some hypoxaemia and respiratory depression was observed. Depression in the heart rate was the most sever side effects. The changes in haematobiochemical findings were mild and transient. Further studies will be required to demonstrate the effectiveness of DKM-3 combination in buffalo calves in clinical settings and field conditions to quantitatively evaluate surgical anaesthesia/analgesia.

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REFERENCES

1. Doherty, T.J., J.A. Ballinger and W.N. McDonnell, 1987. Antagonism of xylazine induced sedation by idazoxan in calves. Can. J. Vet. Res., 51: 244-248.

2. Raekallio, M., M. Kivalo and H. Jalanka, 1991. Medetomidine/ ketamine sedation in calves and its reversal with atipamezole. J. Vet. Anaesth., 18: 45-47.
3. Thompson, J.R., K.W. Kersting and W.H. Hsu, 1991. Antagonistic effect of atipamezole on xylazine-induced sedation, bradycardia and ruminal atony in calves. Am. J. Vet. Res., 52: 1265-1267.
4. Uggla, A. and A. Lindqvist, 1983. Acute pulmonary oedema as adverse reaction to the use of xylazine in sheep. Vet. Rec., 113: 42-47.
5. Thurmon, J.C., G.J. Benson and W.J. Tranquilli, 1986. Cardiovascular effects of intravenous infusion of guaifenesin, ketamine and xylazine in Holstein calves. Vet. Surg.,15: 463.
6. Rings, D.M. and W.W. Muir, 1982. Cardiopulmonary effects of intramuscular xylazine-ketamine in calves. Can. J. Comp. Med., 46: 386-389.
7. Waterman, A.E., 1983. Effect of a combination of ketamine and xylazine on respiratory gas tensions and acid-base status in calves. Vet. Rec., 113: 517-524.
8. Thurmon, J.C., H.C. Lin and G.J. Benson, 1989. Combining telazol and xylazine for anesthesia in calves. Vet. Med., 84: 824-830.
9. Pawde, A.M., P. Amarpal Kinjavdekar, H.P. Aithal, K. Pratap and G.S. Bisht, 2000. Detomidine-diazepam-ketamine anaesthesia in buffalo (Bubalus bubalis) calves. J. Vet. Med Series., A., 47: 175-179.
10. Greenblatt, D.J. and D.R. Abemethy, 1985. Midazolam pharmacology and pharmacokinetics. Anesth. Rev.,12: 17-20.
11. SAS, 2006. Statistical Analysis System.Users Guide: Statistics. SAS Carry Incorporation, North Carolina.
12. Blaze, C.A., R.E. Holland and A.L. Grant, 1988. Gas exchange during xylazine-ketamine anesthesia in neonatal calves. Vet. Surg., 17: 155-159.
13. Bradshaw, C.J., L.W. Traill, K.L. Wertz, W.H. White and I.M. Gurry, 2005. Chemical immobilisation of wild banteng (Bos javanicus) in northern Australia using detomidine, tiletamine and zolazepam. Aust. Vet. J., 83: 616-617.
14. Hofkes, L.M., M.J. Hoyer, P. Van Dijk and P.A. Overgaauw, 2005. Immobilization of cattle and bison with a combination of xylazine, zolazepam-tiletamine and ketamine. Tijdschrift Voor Diergeneeskunde, 130: 268-272.
15. Nuh, K., 2008. Cardiopulmonary, Biochemical and Haematological Changes after Detomidine-Midazolam-Ketamine anaestheia in calves. Bull Vet. Inst. Pulawy., 52: 453-456.
16. Michela, R.E., J. Francisco, I.A. Blanco-Murcia and I.A. Góomez de Segura, 2011. Chemical restraint and anaesthetic effects of a tiletamine-zolazepam/ ketamine/detomidine combination in cattle. The Veterinary Journal, 190: 66-70.
17. Wilson, S.C., D.L. Armstrong, L.G. Simmons, D.J. Morris and T.S. Gross, 1993. A clinical trial using three regimens for immobilizing gaur (Bos Gaurus). J. Zoo. Wildlife Med., 24: 93-101.
18. Caulkett, N., M.R. Cattet, S. Cantwell, N. Cool and W. Olsen, 2000. Anesthesia of wood bison with medetomidine-zolazepam/tiletamine and xylazine-zolazepam/tiletamine combinations. Can. Vet. J., 41: 49-53.
19. Lin, H.C. and M.G. Riddell, 2003. Preliminary study of the effects of xylazine or detomidine with or without butorphanol for standing sedation in dairy cattle. Vet. Ther., 4: 285-291.
20. Singh, P., K. Pratap, P. Kinjavdekar, H.P. Aithal, G.R. Singh and R. Pathak, 2006. Xylazine, ketamine and their combination for lumbar epidural analgesia in water buffalo calves (Bubalus bubalis). J. Vet. Med Series A., 53: 423-431.
21. Verstegen, J., X. Fargetton and F. Ectors, 1989. Medetomidine/ketamine anaesthesia in cats. A Vet. Scand Suppl., 85: 117-123.
22. Nolan, A., A. Livingston and A. Waterman, 1987. Antinociceptive action of intravenous α2-adrenoceptor agonist in sheep. J. Vet Pharmacol. Ther., 10: 202-209.
23. Ponder, S.W. and W.G. Clarke, 1980. Prolonged depression of thermoregulation after xylazine administration to cats. J. Vet. Pharmacol. Ther., 3: 203-207.
24. Macdonald, E., H. Scheinin and M. Scheinin, 1988. Behavioural and neurochemical effects of medetomidine, a novel veterinary sedative. Eur. J. Pharmacol., 158: 119-127.
25. Meyer, H., S.B. Kastner, M. Beyerbach and J. Rehage, 2010. Cardiopulmonary effects of dorsal recumbency and high-volume caudal epidural anesthesia with lidocaine or xylazine in calves. The Veterinary Journal, 186: 316-322.
26. Rioja, E., C.L. Kerr, S.S. Enouri and W.N. McDonell, 2008. Sedative and cardiopulmonary effects of medetomidine hydrochloride and xylazine hydrochloride and their reversal with atipamezole hydrochloride in calves. American Journal of Veterinary Research., 69: 319-329.

27. Koichev, K., D. Golemanov, H. Houbenov and B. Aminokov, 1988. Experimental study on the effect of Domosedan in sheep and cattle. J. Assoc. Vet Anesth., 15: 114-119.
28. Jezek, J., M. Klopcic and M. Klinkon, 2006. Influence of age on biochemical parameters in calves. Bull Vet. Inst. Pulawy., 50: 211-214.
29. Angel, I. and S.Z. Langer, 1988. Adrenergic induced hyperglycaemia in anaesthetized rats: involvement of peripheral α2-adrenoceptors. Eur J. Pharmacol., 154: 191-196.
30. Hsu, W.H. and S.K. Hummel, 1981. Xylazine induced hyperglycaemia in cattle: a possible involvement of α2-adrenergic receptors regulating insulin release. Endocrinology., 109: 825-829.
31. Wagner, A.E., W.W. Muir and K.W. Hinchcliff, 1991. Cardiovascular effects of xylazine and detomidine in horses. Am. J. Vet. Res., 52: 651-657.
32. Kilic, N., 2004. Physiological and haematobiochemical changes in ketamine/diazepam anaesthesia in horse. Indian Vet. J., 81: 396-398.