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Evaluation of xylazine-diazepam-ketamine anaesthesia in mules: Anaesthetic, haemato-biochemical, and blood gas studies

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ABSTRACT

The present study was conducted on 6 clinically healthy adult mules of both sexes (4 females and 2 castrated males), with the approval of IAEC, to evaluate the total intravenous anaesthesia with diazepam-ketamine mixture following xylazine premedication. The anaesthetic combination resulted in quick induction (1.84±0.52 min), prolonged sedation, good to moderate analgesia, moderate muscle relaxation, and smooth and quick recovery. The duration of anaesthesia was 15.21±0.64 min. During recovery, limb and head movements, sternal recumbency, standing ataxia, and normal gait were attained at 16.0±3.21min, 23.30±1.23min, 25.5±3.20 min, and 32.75±3.25 min, respectively, from the time of diazepam-ketamine administration. The anaesthetic combination did not have significant effect on rectal temperature, haemato-biochemical parameters, blood gases, electrolytes, and oxygen status except a significant progressive decrease in the heart rate form the base value up to 15 min after induction, returning to the base level by 45 min, and significant fluctuations in the respiratory rate within the physiological limits. The partial pressure of oxygen in arterial blood decreased to below normal during anaesthesia, however, the oxygen saturation of haemoglobin and oxygen content of blood were well maintained throughout the period of anaesthesia. The combination can be used safely for moderate depth of anaesthesia in mules.

Information is available on equine anaesthesia involving horses and ponies, but very little literature reports about anaesthesia of mules. The results of studies conducted in horses and ponies are extrapolated in mules, invariably resulting in inadequate anaesthesia because there are several anatomical and physiological differences between horses and mule's (Matthews *et al.* 1992). Hence the study was conducted to evaluate the xylazine-diazepam-ketamine total intravenous anaesthesia in mules. A vast literature is available regarding use of these drugs for anaesthesia of horses; however, there are only few published reports about their use in mules.

Adult clinically healthy mules (4 females and 2 castrated males), aged 20±2.5 years and weighing 237.5±20 kg, working at high altitudes of North India, were fasted for 12 h and water was withheld for 6 h before the trials. The animals were premedicated with xylazine hydrochloride @ 1.3 mg/kg intravenously (IV); 5 min after xylazine administration, anaesthesia was induced by administering a mixture of diazepam (0.03 mg/kg) and ketamine hydrochloride (2.2 mg/kg IV). Caution was exercised to prevent forward fall of the animals to avoid head and neck injury. The mules were left undisturbed; and head and neck were extended to maintain a patent airway. Physiological parameters were recorded and blood samples were collected before administration of any drug (0) and at 5, 15 and 45 min after administration of diazepam-ketamine mixture. Blood samples from jugular vein were obtained for estimating differential leucocyte count (DLC), haemoglobin (Hb), packed cell volume (PCV) and total leukocyte count (TLC) as per Jain (1986) and serum was used for estimation of biochemical parameters, viz. glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin (A), total proteins (TPR), blood urea nitrogen (BUN), creatinine and creatine kinase (CK) by using commercially available standard biochemical kits on double beam UV spectrophotometer. Arterial blood samples (1 ml) drawn from caudal auricular artery with 26

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guage needle in pre-heparinzed syringes were used for estimation of various blood gases, electrolytes and oxygen parameters by using automated blood gas analyzer.

The anaesthetic parameters recorded were the time between xylazine administration and development of signs of sedation like drooping of lips, dropping of ears, head down and ataxia; down time—time interval between administration of diazepam-ketamine mixture and attainment of sternal recumbency; induction—time interval between administration of diazepam-ketamine mixture and attainment of lateral recumbency. The quality and depth of anaesthesia was analyzed by recording different reflexes, extent of muscle relaxation and analgesia at 5, 15 and 45 min after the administration of diazepam-ketamine mixture. Presence or absence of palpebral reflex, corneal reflex, swallowing reflex, and response to noise were recorded and graded as 0, 1, 2 and 3 depending on response, where '0' represented absence of response, '3' represented maximum or normal response, and '1' and '2' represented the mild and moderate response, respectively. Muscle relaxation of neck, jaws, tail and anal sphincter were examined and score from 1 to 4 was awarded as per Muir *et al.* (1978) (where 1 indicated 'poor' muscle relaxation evidenced by rigidity in muscles of neck, head and limbs; and contracted anal sphincter; 2 'moderate' - partial relaxation of head, neck and limb muscles and anal sphincter; 3 'good' - adequate muscle relaxation for surgical procedure and 4 'excellent' - complete relaxation). The quality of analgesia was recorded by observing response to noxious stimuli with pin prick at flank region, skin pinch with haemostat at the coronary band and the slap over the rump. Purposeful skeletal muscle movement, observed at any of the test sites, was interpreted as a response and score was given ranging from 0 to 3 (Nanda 2009) (where 0 indicated 'Excellent' analgesia, i.e. no response; 1 'good' i.e. responded to stimuli, nystagmus created; 2 'moderate', i.e. purposeful movement of limbs, head, or neck produced and 3 'poor', i.e. mule moved into sternal position or stood). The time interval between administration of diazepam- ketamine mixture and start of the limb/head movements, turning to sternal recumbency, standing ataxia and normal gait were recorded. Recovery scores ranging from 1 to 5 were given as per Ringer *et al.* (2007). The data generated were analyzed using ANOVA at 5% level of significance.

Table 1. Reflex status at different time intervals following xylazine-diazepam-ketamine anaesthesia in mules (n=6)

Reflexes	Scores (mean±SE) at different time intervals		
	5 min	15 min	45 min
Palpebral	0.66±0.21	0.66±0.33	3.00±0.00
Corneal	1.33±0.55	1.66±0.42	3.00±0.00
Swallowing	0.00±0.00	0.83±0.30	2.66±0.21
Response to noise	0.16±0.16	1.00±0.25	3.00±0.00

Reflexes: 1, 2 and 3 indicate mild, moderate and normal responses, respectively, whereas '0'indicates absence of reflexes.

Table 2. Extent of muscle relaxation at different time intervals following xylazine-diazepam-ketamine anaesthesia in mules (n=6)

Muscle relaxation	Scores (mean±SE) at different time intervals		
	5 min	15 min	45 min
Limbs	2.66±0.21	2.5±0.22	NR
Jaws	1.50±0.22	2.33±0.21	NR
Tail	2.16±0.30	2.00±0.25	NR
Anal sphincter	1.16±0.16	1.00±0.00	NR

Muscle relaxation: 1, poor (rigidity in muscles of neck, head and limbs; and contracted anal sphincter); 2, moderate (partial relaxation of head, neck and limb muscles and anal sphincter); 3, good (adequate muscle relaxation for surgical procedure); 4, excellent (complete relaxation); NR, not recorded as the animals had recovered.

RESULTS AND DISCUSSIONS

All the animals were ataxic at 1±0.5 min following xylazine administration. All the animals were down in sternal recumbency after 1.27±0.52 min of diazepam-ketamine administration and immediately went to lateral recumbency, at 1.84±0.52 min, with initial hypertonicity of hind limbs. The reason for short down time and induction time, initial hypertonicity of limbs is that ketamine, because of its small molecular weight, a pKa near the physiologic pH (7.5) and high lipid solubility, has rapid onset of action, with maximal effect occurring in approximately one minute as reported by Lin (1996). The muscle relaxation could be improved by increasing the dose of diazepam to 0.05–1 mg/kg IV as suggested by Staffieri and Driessen (2007). The results were comparable to that of Kerr *et al.* (1996) who reported recumbency at 0.54±0.21 min after the induction of anaesthesia in horses.

At 5 and 15 min the palpebral reflex was either absent or mild, corneal reflex was mild to moderate, swallowing reflex was absent at 5 and mild at 15 min. The response to noise was mild to moderate at 5 and 15 min. At 45 min the response to all reflexes was strong (normal) except swallowing reflex which was moderate (Table 1). Lacrimation was observed in 1 animal 15 min after diazepam-ketamine mixture administration, whereas nystagmous was seen in 1 animal at 5 min of the diazepam-ketamine administration. However, voluntary blinking, lateral nystagmous and lacrimation have a little value after ketamine administration (Bertone and Horspool 2004).

Anaesthetic combination produced varying degree of muscle relaxation of neck, tail, limbs and jaw after initial hypertonicity (Table 2). Overall muscle relaxation was moderate. The muscle relaxation was attributed to significant muscle relaxing effect of xylazine (Hubbell *et al.* 1989) and

Table 3. Quality of analgesia at different time intervals following xylazine-diazepam-ketamine anaesthesia in mules (n=6)

Quality of analgesia	Scores (mean±SE) at different time intervals		
	5 min	15 min	45 min
Touch/slap	0.50±0.34	1.33±0.42	NR
Skin pinch with hexamostat	0.00±0.00	0.33±0.21	NR
Pin prick	0.33±0.21	0.83±0.30	NR

Quality of analgesia: 0, excellent (no response)*;* 1,good (response to stimuli, nystagmous created)*;* 2,moderate (purposeful movement of limbs*;* head, or neck produced)*;* 3,poor (mule moved into sternal position or stood up)*;* NR,not recorded as the animals had recovered.

diazepam (Matthews *et al*. 2002). Comparatively less complete relaxation of anal sphincter and jaws was observed. The anaesthetic combination did not produce excellent muscle relaxation of any body part at any point of time during anaesthesia. In contrast, Kerr *et al.* (1996) and Thakur *et al.* (2011) reported excellent relaxation of muscles of trunk and limbs in horses and ponies using combination of xylazine and ketamine. In the present study there was poor to moderate relaxation of jaws and anal sphincter at the time interval of 15 min after diazepam-ketamine administration similar to the observation of Muir (1991) in horses.

There was complete to moderate analgesia during anaesthesia in mules which remained for 15.21±0.64 min following the attainment of lateral recumbency. The response to touch/slap had a higher score, when compared to the response to painful stimuli like pin prick and skin pinch throughout the period of anaesthesia (Table 3). The analgesia observed in the present study could be attributed to the combination of stimulation of alpha$_2$ adrenoceptors in CNS by xylazine which inhibits release of neurotransmitter and decrease neuronal activity resulting in loss of pain reflexes (Kinjavdekar *et al.* 1999) and due to ketamine induced blockade of conduction of pain impulses to the thalamic and cortical areas as reported by Booth (1988).

During recovery, limb and head movements; and sternal recumbency were attained in 16.0±3.21 min and 23.30±1.23 min, respectively, from the time of diazepam-ketamine administration. Thus the duration of anaesthesia was 15.21±0.64 min, though with moderate muscle relaxation. Standing ataxia and normal gait were seen at 25.5±3.20 min and 32.75±3.25 min, respectively, from diazepam-ketamine administration. In spite of return of the swallowing reflex, the tongue protrusion was a constant feature during recovery. The recovery was quite and smooth, but animals needed more than one attempt to stand (score 2±0.36). In contrast, longer recovery time was reported by Thakur *et al.* (2011). Our findings were almost similar to the findings of Kerr *et al.* (1996) who observed that the xylazine-diazepam-ketamine combination provided excellent induction and recovery in horses and anaesthesia lasted 15.8l±1.6 min; during recovery, the time to sternal recumbency was 25.67±1.5 min and time to standing was 32.2±3.3 min.

Table 4. Physiological and haemato-biochemical parameters (mean±SE values) following xylazine-diazepam-ketamine anaesthesia in mules (n= 6)

Parameters (unit)	0 min	5 min	15 min	45 min
Rectal temp (°F)	99.43 ±0.85	99.55 ±0.70	98.83 ±0.68	98.97 ±0.54
Heart rate beats/min	58.67 ±2.23^{a}	50.00 ±3.22^{a,b}	45.33 ±1.69^{b}	56.17 ±2.74^{a}
Respiration rate/min	17.83 ±2.31^{a,b}	23.17 ±1.64^{b}	14.67 ±1.98^{a}	16.83 ±1.79^{a,b}
Hb (g/dl)	10.07 ±0.21	11.08 ±0.170	10.50 ±0.42	10.35 ±0.28
PCV (%)	27.0 ±3.68	30.67 ±1.91	29.167 ±2.10	27.67 ±2.09
TLC (×10^3/µl)	7.50 ±0.73	7.87 ±0.70	7.40 ±0.56	7.65 ±0.69
Neutrophil (%)	56.33 ±1.58	56.33 ±0.61	56.50 ±1.26	55.67 ±1.38
Lymphocyte (%)	21.33 ±1.22	23.66 ±0.61	23.50 ±1.26	21.17 ±0.48
Monocyte (%)	12.33 ±0.61	10.33 ±0.93	11.00 ±0.85	12.00 ±0.52
Eosinophil (%)	6.00 ±1.37	8.00 ±0.00	7.00 ±0.44	6.83 ±0.83
Basophil (%)	3.66 ±0.33	2.00 ±0.00	2.33 ±0.33	3.60x ±0.40
Glucose (mg/dl)	79.67 ±5.63	68.37 ±12.34	68.75 ±9.42	70.83 ±8.42
Total protein (g/dl)	6.93 ±0.40	6.38 ±0.55	6.81 ±0.99	6.23 ±0.52
Albumin (g/dl)	2.15 ±0.33	2.41 ±0.96	2.71 ±0.28	2.25 ±0.27
BUN (mg/dl)	7.14 ±2.35	8.03 ±1.85	18.53 ±4.94	9.57 ±2.50
Creatinine (mg/dl)	0.10 ±0.67	0.40 ±0.29	0.39 ±0.25	0.11 ±0.08
CK (IU/L)	31.44 ±3.57	45.76 ±4.86	39.17 ±1.33	37.01 ±4.44
ALT (IU/L)	31.80 ±9.73	24.15 ±8.92	37.88 ±10.64	30.39 ±7.44
AST (IU/L)	247.25 ±17.81	343.05 ±62.67	364.77 ±38.70	371.15 ±70.10

Means bearing different superscripts in a row differ significantly (P<0.05).

The values of physiological and haemato-biochemical parameters are presented in the Table 4. The rectal temperature showed nonsignificant fluctuation within normal range throughout the period of anaesthesia. Initial rise in rectal temperature at 5 min interval might be due to hypertonicity of skeletal muscles. The mules showed a significant (P<0.05) decrease in the heart rate during anaesthesia returning to the base level at 45 min, however,

the changes were within normal physiological range and in agreement with the findings of Kerr *et al.* (1996), Malik and Singh (2007) and Thakur *et al.* (2011). The decrease in the heart rate could be attributed to decreased sympathetic outflow from CNS, vagal activation due to central activation of alpha-2 adrenoceptors by xylazine and involvement of baroreceptor reflex induced by xylazine (Antonaccio *et al.* 1973). The initial significant increase (P<0.05) in respiration rate at 5 min might be due to ketamine induced varying degree of hypertonous and purposeful or reflexive skeletal muscle movements unrelated to surgical stimulus (Lin 1996). The decrease in respiratory rate later though nonsignificant, may be due to direct depressant effect of xylazine on respiration (Muir *et al.* 1977), due to muscle relaxation by diazepam and xylazine, and depth of anaesthesia, caused by synergistic action of diazepam, xylazine and ketamine (Malik and Singh 2007), although ketamine by itself does not cause respiratory depression, rather it may return respiratory rate to pre-xylazine values.

The Hb, PCV, TLC and DLC showed nonsignificant variations and remained within normal range following the use of xylazine-diazepam-ketamine combination for TIVA in mules. The values observed during anaesthesia and in post anaesthetic period were comparable to the base values. This indicated that there was neither any sequestration of red blood cells and white blood cells in the spleen, liver etc. nor shifting of fluid between the compartments (Thakur *et al.* 2011). The base value of monocyte count was towards the higher limit of the normal range, which might be due to the stress of working under harsh climatic conditions at high altitudes.

Generally observed hyperglycemia following the xylazine administration due to an $alpha_2$ adrenergic inhibition of insulin release by stimulation of $alpha_2$ receptors in the pancreatic *b* - cells (Angel and Langer 1988) and also due to the increased glucose production in the liver (Hsu and Hummel 1981) was not observed in the present study.

Serum total protein and albumin showed a nonsignificant alteration in the present study. The BUN, creatinine and CPK fluctuated within the normal physiological range throughout the period of study as also observed by Hikasa *et al.* (1994) in atropine-xylazine-guaifenesin-thiopentone anaesthesia in spontaneously breathing horses, indicating that renal blood flow was maintained normally and lack of any post-anaesthetic myopathy. The alterations in ALT and AST were nonsignificant and within normal range in agreement with Malik and Singh (2007).

The blood gases, oxygen and electrolyte status are presented in Table 5. The alterations in the arterial blood pH and stpH in all the animals recorded at different intervals were nonsignificant (P>0.05). Muir *et al.* (1982) concluded that administration of diazepam to horses produces negligible effect upon haemodynamic, pH, and blood gases. Similarly, Ellis *et al.* (1997) reported that pH and blood gases remained within the physiological ranges after administration of xylazine and ketamine in horses. The partial pressure of carbon dioxide in arterial blood ($PaCO_2$) of the mules undergoing anaesthesia recorded a nonsignificant alteration throughout the study period and remained within normal range of 40.0±3.00 mmHg (Muir and Hubbell 1995). $PaCO_2$ increased from 38.66±0.95 mmHg at 0 min to 39.33±0.67 mmHg at 45 min after an initial decrease to 37.67±1.08 mmHg at 5 min. Initial fall in $PaCO_2$ at 5 min could be due to increased respiratory rate seen after 5 min produced due to hypertonicity of muscles as a result of ketamine administration (Lin 1996). Slight increase in $PaCO_2$ values after the initial decrease suggested that there was some degree of hypoventilation, as there is lack of a compensatory increase in alveolar ventilation during sedation with $alpha_2$ agonist like xylazine (Nyman *et al.* 2009). The partial pressure of oxygen in arterial blood (PaO_2) decreased to below normal 80 to 110 mmHg (Haskins 1996) during anaesthesia, however, it returned to normal by 45 min. There was a severe fall in PaO_2 from the base value of 85.66±1.91 to 69.17±5.25 mmHg, though the fall was statistically nonsignificant. Initial fall in PaO_2 could be attributed to attainment of lateral recumbency as horses when placed in lateral recumbency under injectable anaesthesia generally show marked fall in PaO_2 and mild increase in $PaCO_2$ (Muir *et al.* 1977). Our findings are in agreement with the observations of Hubbell *et al.* (1989) who reported a significant reduction in the mean PaO_2 (P<0.01) few min after ketamine induction in horses. They also reported that this hypoxemia persisted throughout the study. However, in the present study the oxygen saturation of haemoglobin and oxygen content of blood showed a non-

Table 5. Blood gases, oxygen status and electrolytes (Mean±SE) following xylazine-diazepam-ketamine anaesthesia in mules (n=6)

Parameters (unit)	0 min	5 min	15 min	45 min
pH	7.46±0.07	7.47±0.02	7.47±0.01	7.47±0.02
stpH	7.45±0.04	7.45±0.01	7.46±0.00	7.46±0.00
cH^+ (nmol/L)	34.90±0.54	34.18±0.59	35.65±2.01	34.15±0.50
$PaCO_2$ (mmHg)	38.66±0.95	37.67±1.08	39.17±1.83	39.33±0.67
PaO_2 (mmHg)	85.66±1.91	69.17±5.25	72.50±7.68	80.33±2.54
HCO_3^- (mmol/L)	28.35±2.00	26.55±0.47	27.63±0.72	27.70±0.60
BE (mmol/L)	2.72±0.31	2.80±0.36	3.75±0.69	3.77±0.06
$stHCO_3^-$ (mmol/L)	26.58±0.26	22.65±0.29	27.47±0.58	27.47±0.51
SaO_2 (%)	95.50±0.56	91.67±1.47	91.50±1.78	93.66±0.76
O_2ct (vol.%)	13.65±0.32	13.85±0.20	14.43±0.48	14.40±1.28
Sodium (mmol/L)	128.0 ±2.17	126.67 ±1.63	124.50 ±1.15	127.16 ±1.90
Potassium (mmol/L)	7.12±1.05	4.84±0.67	5.81±1.07	7.78±0.75
Calcium (mmol/L)	3.05±0.11	2.84±0.04	2.60±0.10	2.7±0.88

Means in a row do not differ significantly (P>0.05).

significant alterations fluctuating within the normal range. Arterial oxygen content (O_2ct vol.%) was well maintained in all the animals showing slight increase after induction relative to the baseline. Arterial haemoglobin values paralleled this increase and could be responsible for increased oxygen carrying capacity in this study. However, Frias *et al.* (2003) reported the decrease in oxygen content of blood after induction with the different doses of propofol in xylazine premedicated horses. The values of oxygen saturation of haemoglobin (SaO_2%) in present study showed only a slight nonsignificant change and remained slightly below the base values of 95.50±0.0.56% during the entire period of study. The changes were correlated with changes observed in PaO_2 as the 2 are closely related to each other, as pH decreases and the $PaCO_2$ and the local temperature increases, at any given PaO_2 value, haemoglobin releases oxygen to surrounding environment more rapidly as reported by McDonell (1996). Thus the combination of xylazine-diazepam-ketamine in mules induced slight hypoventilation, as evidenced by an increase in $PaCO_2$ and marked decrease in PaO_2, however, the lungs were able to deliver oxygen to maintain SaO_2 and O_2ct at almost base level, not requiring respiratory support.

The bicarbonates (HCO_3^-) levels at 0, 5, 15 and 45 min were 28.35±2.00, 26.55±0.47, 27.63±0.72, and 27.70±0.60 mmol/L, respectively, and found to be within normal range for horses (Muir and Hubbell 1995). The alterations in the levels of bicarbonate correlated with the levels of $PaCO_2$ as both the parameters decreased at 5 min after the administration of diazepam-ketamine followed by slight increase at 15 min interval. The slight increase in bicarbonate value at 15 min after the initial decrease at 5 min could be attributed to increased level of carbon dioxide ($PaCO_2$) in the blood which leads to the increase in production of carbonic acid. Watson *et al.* (2002) reported an increase in bicarbonate after induction of general anaesthesia in horses. There was a progressive increase in base excess (BE) mmol/L throughout the period of study and was comparable to that obtained after the use of xylazine @ 1.1 mg/kg, diazepam @ 0.04 mg/kg and ketamine @ 2.2 mg/kg intravenously by Kerr *et al*. (1996) in horses. The nonsignificant increase was observed in saturated concentration of bicarbonates ($stHCO_3^-$) at 15 and 45 min after the initial decrease at 5 min following diazepam-ketamine administration. The increase in $stHCO_3^-$ may be attributed to similar reasons as to increase in concentration of HCO_3^-.

The nonsignificant decrease in the mean of serum sodium, potassium and calcium was observed during the period of study and was in agreement with Malik (2007) who reported nonsignificant decrease in serum sodium and potassium at sedation and at maximal depth of anaesthesia and touched the base line values within 12 h; and Grubb *et al.* (1997) who observed non-significant change in serum ionized and total calcium after administration of xylazine and ketamine anaesthesia in horses.

Thus, TIVA using xylazine-diazepam-ketamine protocol resulted in quick induction, prolonged sedation, moderate analgesia and muscle relaxation; and smooth rapid recovery. The anaesthetic regimen did not influence the blood gases, electrolytes, and oxygen status significantly (P>0.05). Combination of xylazine-diazepam-ketamine can be safely used for short term anaesthesia of moderate depth in mules.

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