Evaluation of some anaesthetic protocols for induction of anaesthesia in donkeys (Equus asinus) in Sudan

J. A. Abakar (1), M. A. H. Ghurashi (2), H. I. Seri (3)*

1 Faculty of Veterinary Science, University of Nyala
2 Open University of Sudan
3 College of Veterinary Medicine, Sudan University of Science and Technology.

* Corresponding Author: College of Veterinary Medicine, P.O. Box: 204 Khartoum North, Email: hishamseri@sustech.edu
Article history: Received: 22.2.2014
Accepted: 08.03.2014

Abstract

The aim of this study was to determine an appropriate combination of Xylazine, Ketamine, and Diazepam (XKD) that would produce safe and satisfactory total intravenous anaesthesia in donkeys, for use under field conditions. In this study three protocols containing Ketamine (3 mg/kg), Xylazine (1.5 mg/kg) and diazepam at (0.1, 0.2 and 0.3 mg/kg) were tested. A total of 18 donkeys, twelve male and six female was used. The animals were allocated randomly to three groups of equal size and each group was anaesthetized with a different anaesthetic protocol. Respiratory rate, heart rate and rectal temperature were measured at 10 minutes interval before, and throughout the duration of anaesthesia. Anaesthesia parameters (induction time, duration of anaesthesia, lateral recumbency, sternal recumbency, standing, and total recovery time) were measured and recorded at 10 minutes interval post induction throughout the duration of anaesthesia. Quality of induction, recovery and muscle relaxation was also evaluated subjectively during anaesthesia. All anaesthetic protocols used produced satisfactory induction of anaesthesia and quite smooth recovery and non significant increase in the duration of anaesthesia phase was observed. While, significant increase in the duration (P < 0.05) of lateral recumbency, sternal recumbency, standing position, total recovery time, and reflexes was observed. Induction of anaesthesia, muscle relaxation and recoveries were acceptable in all protocols used. It is to be concluded that these protocols were proved to be safe for use under field conditions for donkeys.

Keywords: Total intravenous anaesthesia, Ketamine, Xylazine, Diazepam, Donkeys.

Introduction

Differences in drug kinetics as well as behavioural differences between donkeys and horses seem to make it difficult to find the optimal field anaesthetic in donkeys (Matthews and Taylor, 2000). Numerous combinations of agents have been employed in equine for induction of anaesthesia. Xylazine/ketamine (X/K) was the first reported combination of this type (Muir et al., 1977) and remains the standard field
anaesthetic technique in North America (Muir, 1991).
Cardiopulmonary investigations involving such combinations have been published and have shown favourable responses compared to the previous barbiturate-based anaesthetic regimes (Hubbell et al., 1989; Radi et al., 2011; Radi et al., 2012). Diazepam ketamine combinations have been suggested as an alternative induction technique to short acting barbiturates like thiopentone (Hellyer et al., 1991). In the horse Diazepam is given at a dose of 0.05 to 0.1 mg/kg IV immediately before or in combination with the standard dose of Ketamine (2.2 mg/kg IV). The addition of diazepam produces improved quality of anaesthesia without compromising the cardio-respiratory system and increases the duration of anaesthesia from 20 to 25 minutes (Hubbell, 1999).

In donkeys, higher doses of Xylazine have been recommended than what is normally used in the horse, the doses of Xylazine normally used are in the range of 0.5 -2mg/kg (Mbiuki and Mogoa, 1994; and Mogoa et al., 1994). Addition of midazolam (0.06 mg/kg) or diazepam (0.03 - 0.06 mg/kg) therefore is recommended to provide better sedation and muscle relaxation (Matthews and Van Dijk, 2004).

In donkeys, to our knowledge, few or no adequate research work pertaining to injectable anaesthesia, had been done, and usually donkeys are considered like horses although they seem slightly different, therefore this study was conducted to determine a satisfactory combination of diazepam, ketamine, and xylazine that would produce safe and acceptable total intravenous anaesthesia in donkeys to be used under field conditions.

Materials and methods
Place of study: This study was carried out at the College farm, College of Veterinary Medicine, Sudan University of Science and Technology, Khartoum North, Hillat Kuku, Sudan.

Experimental animals: A total number of 18 adult donkeys (12 male and 6 females) was used in this study. Their mean age was 4.8±1.2 years and mean weight was 94.2±20.4kg. Animals were clinically examined to ascertain freedom from diseases and they were treated with Albendazole at 10mg/kg; and Penicillin G Procaine and Dihydrostreptomycin Sulfate Injection (Pen Strep 1ml/20kg) as prophylactic treatment and kept for 15 days as adaptation period. Animals were randomly allocated into three treatment groups. They were fed twice a day and they were allowed with free access to water.

Drugs: One anaesthetic agent and two pre-anaesthetic medications were used in this study, as follows:
1. Ketamine Hcl 5% (Troika pharmaceuticals Ltd Th-382728 Gujarat, India).
2. Xylazine Hcl (Rompun 2%, alfasan, Holland).
3. Diazepam (0.5%) (Shanghai pharmaceutical company (Shaphar), China).

Injection set: Disposable syringes 5, 10 and 20 (Nirma limited health care division Sachana Gujarat 382150, India), and intravenous catheters (18 G) were used for intravenous injection of drugs.

Pilot study: Six donkeys were used in a pilot trial to evaluate response of animals to xylazine with ketamine. The first two animals were anaesthetized with 1.1 mg/kg xylazine and 2.2 ketamine; another two donkeys were anaesthetized with 1.5 mg /kg xylazine and 3mg/kg ketamine, and the remaining two donkeys were anaesthetized with 2 mg /kg xylazine and 4 mg/kg ketamine.
Experimental design:
Donkeys in the first group were anaesthetized with xylazine 1.5mg/kg, Diazepam 0.1 and Ketamine 3mg/kg, the second group was anaesthetized with xylazine 1.5mg/kg + Diazepam 0.2mg/kg and ketamine 3mg/kg. The third group was anaesthetized with xylazine 1.5mg/kg + Diazepam 0.3mg/kg and ketamine 3mg/kg. In all animals, the anaesthetic drugs were administered through intravenous catheter. Clinical parameters were recorded immediately before injection of the premedicant (baseline values), and immediately following induction of anaesthesia at zero time and at interval of 10 minutes.

Quality of induction of anaesthesia
The quality of induction of anaesthesia was rated as follows:
Satisfactory: rapid and smooth with little danger to animal or personnel (Matthews et al., 2002).
Unsatisfactory; prolonged period of incoordination muscle fasciculation (Matthews et al., 2002).

Quality of muscle relaxation
A score card for the quality of muscle relaxation was used as follows:

<table>
<thead>
<tr>
<th>Score</th>
<th>Quality</th>
<th>Character</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Excellent</td>
<td>Complete relaxation</td>
</tr>
<tr>
<td>3</td>
<td>Good</td>
<td>Adequate muscle relaxation for surgical procedure</td>
</tr>
<tr>
<td>2</td>
<td>Moderate</td>
<td>Partial relaxation in muscles of head, neck and limb</td>
</tr>
<tr>
<td>1</td>
<td>Poor</td>
<td>Rigidity in muscles of neck, head and limbs</td>
</tr>
</tbody>
</table>

Quality of recovery
A score, ranging from 1 to 5 as per method of Ringer et al., (2007) was used for the assessment of quality of recovery from anaesthesia as follows:

<table>
<thead>
<tr>
<th>Score</th>
<th>Quality</th>
<th>Character</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Excellent</td>
<td>Donkey capable of standing at first attempt</td>
</tr>
<tr>
<td>2</td>
<td>Very good</td>
<td>Donkey remained calm and needed two attempts to stand</td>
</tr>
<tr>
<td>3</td>
<td>Good</td>
<td>Donkey remained calm but needed more than two attempts to stand</td>
</tr>
<tr>
<td>4</td>
<td>Poor</td>
<td>Excitement during recovery with danger of injury and needed more than two attempts to stand</td>
</tr>
<tr>
<td>5</td>
<td>Very poor</td>
<td>Severe excitement during recovery with injury</td>
</tr>
</tbody>
</table>

Physiological parameters
Respiratory rate, heart rate and rectal temperature were monitored before injection and immediately after induction of anaesthesia, and at 10 minutes interval following induction of anaesthesia, using standard methods (Kelly, 1984)

Anaesthesia phases
The animals were monitored closely and remarks were reported immediately and phases of anaesthesia were determined as follows:
Induction phase: It is the time elapsed following injection of the anaesthetic and the state or condition in which the animal becomes unconscious, respond negatively to painful stimuli with disappearance of selected reflexes (Jani et al., 1982)
Anaesthetic phase: this is considered as the period during which the animal shows signs of unconsciousness, no reflexes, responds
negatively to painful stimuli (Tamisto et al., 1981).

**Basal narcosis:** this is considered as the period during which the animal shows signs of unconsciousness, but responds positively to noxious or painful stimuli (Atkinson et al., 1987).

**Lateral recumbency:** this is considered as the duration at which the reflexes are regained but it is incapable of adopting sternal position (Thurmon et al., 1996).

**Sternal recumbency:** It is considered as the period during which the animal could adopt sternal recumbancy without falling to lateral recumbancy and without adopting standing position (Ghurashi et al., 2008)

**Standing phase:** It is the stage at which the animal stood but unable to walk ten steps (Ghurashi, 1999).

**Recovery:** The animal was considered to be recovered from anaesthesia when it is capable of supporting itself in standing position and walk for ten steps without falling down (Ghurashi et al., 2008).

**Total recovery:** Total recovery time was considered as the total time calculated from the time of induction of anaesthesia until recovery was attained (Nuha, 2004).

### Reflexes

The following reflexes were monitored closely and remarks were reported immediately as follows:

**Tongue reflex:** It was assessed by pulling the tongue outside the mouth, when the animal was capable to pull its tongue into the mouth, the reflex was considered positive (Nuha, 2004).

**Palpebral reflex:** The reflex was assessed by digital touch on the canthus or eyelashes, if purposeful motor reflex observed, the reflex was considered positive (Batoul, 1990).

**Swallowing reflex:** External digital pressure on the larynx was used to assess swallowing reflex. Positive response was considered when swallowing or laryngeal movements were observed (Rawling and Kolata, 1983).

**Jaw relaxation:** Persistence of open mouth due to induced jaw retraction was considered to be a positive jaw relaxation reflex. The reflex was considered regained when the animal was reluctant to open its mouth (Subjective).

**Pedal reflex:** Pedal reflex was assessed by pinprick on the coronary band of the digit. If the animal moves its leg or leg muscle, the reflex was considered positive (Williams and Wyatt, 2007).

**Anal reflex:** Anal reflex was assessed by inducing tension of anal sphincter with two fingers. Positive response was considered when the movement of the anal sphincter was noticed (Subjective).

**Cough reflex:** Gentle digital pressure on the first 3rd tracheal cartilage was used to assess cough reflex. Positive response was considered when the cough was induced (Radostits et al., 2007).

### Statistical analysis

T- Test was used to compare data for physiological parameters, while ANOVA was used to compare between the different anaesthetic phases and reflexes. The Statistical Package for Social Sciences (SPSS) was used to perform these analytical operations. No statistical evaluation of subjective data (i.e. analgesia, muscle relaxation and recovery) was performed.

### Results

**Pilot Study**

In the pilot study, donkeys showed ataxia, excitation and muscle rigidity in the animals anaesthetized with 1.1mg/kg xylazine and 2.2 mg/kg ketamine, while in the two animals anaesthetized with 1.5mg/kg xylazine and 3mg/kg ketamine or 2mg/kg xylazine and 4.4mg/kg ketamine the result was very short period of anaesthesia (less than 5minutes) accompanied by excitation and muscle rigidity.
Signs and observations following injection of premedication

Immediately, following intravenous injection of Xylazine in donkeys, main signs observed were dropping of the head, lowering the lip, ears, abduction of the legs and protrusion of penis (Figure 1), sometimes ataxia and the animal fell down or adopt sterna recumbancy.

Figure 1: Abduction of legs and protrusion of penis following injection of Xylazine

Signs and observations following injection of Ketamine: In all protocols used animals smoothly laid down on the ground and adopt lateral recumbancy, some of them laid down in characteristic way of Ketamine (Figure 2), and eyes remained open (Figure 3).

Figure 2: Donkey assuming dog sitting position following injection of ketamine

Figure 3: Eyes remained open in a donkey following injection of ketamine
Quality of induction, muscle relaxation and recovery

Quality of induction in the three protocols used is satisfactory and muscle relaxation ranged between excellent to very good. As shown in Table (8) recovery phase showed variation between the different three protocols. In the first protocol 16.7% (1 out of the 6 animals) exhibited excellent recovery and the animal stood up in the first attempt and 50% (3 out of the 6 animals) showed very good recovery and the animal needs two attempts with help to stand and 33.7% (2 out of 6) of the animals the recovery was good. Although 16.7% (1 out of six animals) in group 3 showed excitation during anaesthesia, animals anaesthetized with second protocol and third protocol showed good recovery.

Table 1: Recovery grades of three tested protocols

<table>
<thead>
<tr>
<th>Protocols</th>
<th>No. Of animals</th>
<th>Very poor</th>
<th>Poor</th>
<th>Good</th>
<th>Very good</th>
<th>Excellent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>XKD (0.1)</td>
<td>6</td>
<td>2</td>
<td>33.7</td>
<td>3</td>
<td>50</td>
<td>1</td>
</tr>
<tr>
<td>XKD (0.2)</td>
<td>6</td>
<td>6</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XKD (0.3)</td>
<td>6</td>
<td>6</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

X=xylazine, K= ketamine, D=diazepam
Number in brackets indicates the dose of diazepam

Effects of the different protocols on physiological parameters

Respiratory rate: Animals anaesthetized with the first protocol showed significant (P≤0.05) decrease in respiratory rate at time points 10, 20, 30, 40 and 50 minutes following induction and a non significant (P≥0.05) decrease at 0 time when compared with baseline value. In the animals anaesthetized with the second protocol, respiratory rate was significantly decreased (P≤0.05) throughout the duration of anaesthesia. While in animals anaesthetized with the third protocol, respiratory rate showed significant decrease (P≤0.05) at time 10 and 50 minute (Table, 2).

Table 2: Effect of the three tested protocols on respiratory rate (breath/minute) in donkeys

<table>
<thead>
<tr>
<th>Protocols</th>
<th>Baseline</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>XKD (0.1)</td>
<td>28.2±1.9</td>
<td>20.8±5.9</td>
<td>18.8±1.0*</td>
<td>19.8±4.5*</td>
<td>20.8±1.0*</td>
<td>20.5±1.9*</td>
<td>19.8±3.6*</td>
</tr>
<tr>
<td>XKD (0.2)</td>
<td>32.2±2.9</td>
<td>21.0±4.4*</td>
<td>16.2±2.6*</td>
<td>21.2±1.6*</td>
<td>20.8±0.9*</td>
<td>21.5±1.6*</td>
<td>22.0±1.6*</td>
</tr>
<tr>
<td>XKD (0.3)</td>
<td>28.5±1.7</td>
<td>29.0±6.9</td>
<td>17.0±1.7*</td>
<td>24.8±5.2</td>
<td>27.8±5.7</td>
<td>26.3±3.4</td>
<td>21.7±2.7*</td>
</tr>
</tbody>
</table>

Number in brackets indicates the dose of diazepam
Values are mean ± Standard error of mean of six replicates each
Values with superscript within raw are significantly (P ≤ 0.05) different from baseline value

Heart rate: Animals anaesthetized with first protocol, showed significant (P≤0.05) increase in heart rate at 50 minutes and a non significant (P≥0.05) changes at time points 0 and 10, and a non significant increase in 20, 30 and 40 minutes (Table, 3). Animals anaesthetized with the second protocol, revealed no significant (P≥0.05) increase in heart rate throughout the duration of anaesthesia. While in animals anaesthetized with the third protocol, donkeys expressed no
significant increase in heart rate at time points 0, 10, and 30 and a non significant decrease at 20 and 40 minutes compared with baseline value.

Table 3: Effect of the three tested protocols on heart rate (beat/minute) in donkeys

<table>
<thead>
<tr>
<th>Protocols</th>
<th>Baseline</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>XKD (0.1)</td>
<td>44.8±5.1</td>
<td>44.8±2.7</td>
<td>43.7±2.9</td>
<td>49.0±3.5</td>
<td>52.5±5.2</td>
<td>53.7±4.1</td>
<td>63.6±3.2*</td>
</tr>
<tr>
<td>XKD (0.2)</td>
<td>45.0±2.6</td>
<td>58.5±4.9</td>
<td>49.8±3.1</td>
<td>46.7±2.6</td>
<td>54.5±4.6</td>
<td>47.7±2.8</td>
<td>47.2±3.0</td>
</tr>
<tr>
<td>XKD (0.3)</td>
<td>45.2±4.6</td>
<td>50.8±3.6</td>
<td>46.5±3.4</td>
<td>43.2±0.8</td>
<td>47.5±2.5</td>
<td>44.7±1.9</td>
<td>45.0±3.9</td>
</tr>
</tbody>
</table>

Number in brackets indicates the dose of diazepam
Values are mean ± Standard error of mean of six replicates each
Values with superscript within raw are significantly (P ≤ 0.05) different from baseline value

Rectal temperature: As illustrated in Table (4), animals anaesthetized with the three tested protocols, revealed no significant change in rectal temperature during anaesthesia.

Table 4: Effect of the three tested protocols on rectal temperature (°C) in donkeys

<table>
<thead>
<tr>
<th>Protocols</th>
<th>Baseline</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>XKD (0.1)</td>
<td>37.1±0.3</td>
<td>37.0±0.2</td>
<td>36.8±0.3</td>
<td>36.6±0.4</td>
<td>36.8±0.6</td>
<td>36.5±0.4</td>
<td>36.5±0.5</td>
</tr>
<tr>
<td>XKD (0.2)</td>
<td>38.1±0.4</td>
<td>38.1±0.3</td>
<td>38.1±0.3</td>
<td>37.8±0.3</td>
<td>37.9±0.5</td>
<td>37.6±0.4</td>
<td>37.5±0.2</td>
</tr>
<tr>
<td>XKD (0.3)</td>
<td>37.3±0.2</td>
<td>37.5±0.2</td>
<td>37.6±0.3</td>
<td>37.2±0.3</td>
<td>36.9±0.2</td>
<td>36.8±0.3</td>
<td>36.9±0.6</td>
</tr>
</tbody>
</table>

Number in brackets indicates the dose of diazepam
Values are mean ± Standard error of mean of six replicates each
Values with superscript within raw are significantly (P ≤ 0.05) different from baseline value

Duration of different anaesthetic phases following induction of anaesthesia

Results obtained in this study, as shown in Table (5) revealed a non significant (P>0.05) differences in the onset of anaesthesia between the three different anaesthetic protocols used. Also we could observe that in the three tested protocols for induction of anaesthesia, increasing the dose of diazepam resulted in non significant (P>0.05) increase in the anaesthesia phase.

In the third protocol, there was increase in the lateral recumbancy duration which was found to be significantly longer when compared with other two protocols. The increase in the diazepam dose from 0.1 to 0.2 and 0.3 resulted in significant (P<0.05) difference in the duration of sternal recumbency between the first protocol and the third protocol and second protocol protocols. While there was no significant difference between second protocol and third protocol.

Also we could observe a significant (P<0.05) increase in the duration of standing phase between the first protocol and the third protocol, but no significant (P>0.05) difference was observed between the second protocol and the other protocols used.
Table 5: Duration of the different anaesthetic phases following induction of anaesthesia using xylazine, Ketamine and diazepam in donkeys

<table>
<thead>
<tr>
<th>Protocols</th>
<th>Onset</th>
<th>Anaesthetic</th>
<th>Lateral</th>
<th>Sternal</th>
<th>Standing</th>
<th>Total recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>X(K and D 0.1)</td>
<td>1.1±0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.3±1.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.2±1.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.8±1.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.5±0.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>47.0±3.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>X(K and D 0.2)</td>
<td>1.1±0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.8±0.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.7±2.5&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>12.5±1.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.0±0.6&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>54.7±4.1&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>X(K and D 0.3)</td>
<td>1.0±0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.2±1.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.8±3.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13.7±0.7&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>10.7±0.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>60.0±2.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Number in brackets indicates the dose of diazepam
X= Xylazine, K=Ketamine, D=Diazepam
Values are mean (min) ± Standard error of mean of six replicates
Values with different superscript within column are significantly (P ≤ 0.05) different.

Total recovery was significantly (P<0.05) different between the second and both the first and the third protocols (Table, 6).

Table 6: duration of Total recovery time (TRT) following administration of different doses of diazepam with ketamine

<table>
<thead>
<tr>
<th>Protocols</th>
<th>X(K and D 0.1)</th>
<th>X(K and D 0.2)</th>
<th>X(K and D 0.3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total recovery time</td>
<td>47.0±3.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>54.7±4.1&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>60.0±2.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean (min) ± Standard error of mean of six replicates each
X= Xylazine, K=Ketamine, D=Diazepam
Values with different superscript within column are significantly (P ≤ 0.05) different.

The effect of different anaesthetic protocols on selected reflexes:
In this study the palpebral and corneal reflexes were found to be depressed for short 83.3% of the anaesthetized animals in each protocol (Table, 7). Also we could observe that Pedal and anal reflexes were not affected in three protocols used.

Table 7: State of eye reflexes in the three tested protocols

<table>
<thead>
<tr>
<th>Protocols</th>
<th>No of animals</th>
<th>Palpebral</th>
<th>Corneal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td>XKD(0.1)</td>
<td>6</td>
<td>2</td>
<td>33.7</td>
</tr>
<tr>
<td>XKD(0.2)</td>
<td>6</td>
<td>5</td>
<td>83.3</td>
</tr>
<tr>
<td>XKD(0.3)</td>
<td>6</td>
<td>5</td>
<td>83.3</td>
</tr>
</tbody>
</table>

As shown in Table (8), significant (P<0.05) increase in the mean time of Spinal, tail reflexes and jaw relaxation between the first and the third protocol, while no significant (P>0.05) differences between animals anaesthetized with second protocol and both groups of animals anaesthetized with the first and the third protocol.

Also the study revealed that increasing the dose of diazepam (0.1, 0.2 and 0.3 mg/kg) in
combination with Ketamine for induction of anaesthesia resulted in no significant (P>0.05) increase in the mean time of tongue reflex between the first and the second protocol while a significant (P< 0.05) was noticed only following increasing the dose to 0.3mg/kg diazepam.

Table 8: The effect of different anaesthetic protocols on selected reflexes

<table>
<thead>
<tr>
<th>Protocols</th>
<th>Spinal</th>
<th>Tongue</th>
<th>Pedal</th>
<th>Jaw relaxation</th>
<th>Tail</th>
<th>Anal</th>
</tr>
</thead>
<tbody>
<tr>
<td>XKD0.1mg/kg</td>
<td>22.3±1.0a</td>
<td>20.5±0.2a</td>
<td>NA</td>
<td>17.9±0.7a</td>
<td>21.3±2.0a</td>
<td>NA</td>
</tr>
<tr>
<td>XK D0.2mg/kg</td>
<td>25.5±0.7ab</td>
<td>23.2±1.0ab</td>
<td>NA</td>
<td>21.5±1.3ab</td>
<td>23.7±0.2ab</td>
<td>NA</td>
</tr>
<tr>
<td>XKD 0.3mg/kg</td>
<td>28.2±1.2b</td>
<td>26.8±1.7c</td>
<td>NA</td>
<td>25.2±2.0b</td>
<td>27.3±1.6b</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA = not affected
Values are mean (min) ± Standard error of mean of six replicates each
X= Xylazine,  K=Ketamine.  D=Diazepam
Values with different superscript within the same column are significantly (P ≤ 0.05) different.

Discussion

Ketamine had come into the foreground especially during the last decade due to prevention of its side effects by using drug combinations and thus expansion of its area of usage (Akeson et al., 1993). Diazepam in combination with ketamine induces anaesthesia by its action on the part of the limbic system; thalamus and hypothalamus resulting in calming effect (Swindle et al., 2002). The pilot study performed prior to conduction of the current work, revealed that local donkeys in Sudan, responds poorly to xylazine and ketamine, they showed lots of muscle rigidity and excitatory effects, there are several possible explanations for this poor response to ketamine and xylazine, metabolic rate might be higher in miniature donkeys, as they are smaller and should have a higher body surface area (Henness et al., 1977). The duration of anaesthesia was controlled by plasma half life of Ketamine and the quick recovery from Ketamine anaesthesia was due to the redistribution from the central nervous system to other tissues (Wright, 1982). Differences in biotransformation, protein binding and interspecies differences in entero-hepatic circulation are some of the other factors which affect drug disposition. Differences in the pharmacokinetics of caffeine (a capacity limited drug) between donkeys and horses, suggests that there may be differences in cytochrome P450 isoenzymes (Peck et al., 1997). Addition of midazolam or diazepam is recommended to provide better sedation and muscle relaxation (Matthews and Van Dijk, 2004). Therefore depending on the pilot study the dose of ketamine and xylazine was adjusted to 1.5 mg/kg Xylazine and 3 mg/kg Ketamine.

This study revealed that the induction of anaesthesia using ketamine and diazepam was smooth in donkeys, with no apparent danger to animal or personnel. None of the ataxia reported with induction using different doses of diazepam with ketamine, including shifting of weight and fetlock flexion, was seen in this study, this result was supported by (Brock and Hildebrand, 1990; Caulkett, 2007) which encouraged us to suggest that these protocols would be a satisfactory method of inducing total intravenous anaesthesia in donkeys.

The reduction of the respiratory rate observed in this study, was supported by (Haskins et al., 1986), who stated that the decrease in respiratory rate during anaesthesia could be
due to the effect of diazepam which potentiate the respiratory depressant effect of ketamine, and also this respiratory depression is likely the result of a combination of sedation produced by the drugs and the effect of recumbency (Matthews et al. 1999; Taylor and Clarke, 2007).

In this study the heart rate and rectal temperature showed no significant changes in all protocols used except at 50 minutes post induction when the second protocol was used, where there was significant increase in heart rate, and this might be due to panic produced by diazepam as result of muscle relaxation (Doherty and Valverde, 2006). The obtained result is supported by Marntell and Nyman (1996) who stated that, the induction of anaesthesia with diazepam and ketamine or its isomer normalized the heart rate of horse, also this finding is in the same line with previous reports of Koshy et al., (2003) and Davison et al., (2007), who reported a non significant effect of Ketamine on these two parameters. Diazepam had no significant effect on heart rate (Muir and Mason, 1993; and Ghurashi et al., 2009). Diazepam was reported to moderate the cardio stimulatory property of ketamine and maintained the heart rate (Jackson et al., 1978).

This study showed a non significant increase in the duration of the anaesthesia phase in the three anaesthetised groups; this increase may be attributed to the increase in the dose of diazepam. This result is supported by the findings of (Hubbell, 1999; Hall et al., 2001; Caulkett, 2007) another study conducted in humans reported that concurrent administration of diazepam can prolong the plasma half-life of ketamine and its metabolites due to inhibition of hepatic metabolism (Lo and Cumming, 1975), and the same previous reason may support the significance differences in the lateral, sternal recumbancy and recovery times according to the fact that diazepam has a dose dependant effect on central nervous system and muscle relaxation (Muir and Mason, 1993) and the muscle relaxant effects are more prolonged than unconsciousness which may lead to prolongation on previous stages.

In sheep, Lin et al., (1994) investigated the effects of two different anaesthetic regimens, namely ketamine-diazepam and ketamine - diazepam-xylazine combinations and found that the latter combination resulted in a prolonged duration of anaesthesia. In this study, muscle relaxation ranged between excellent to very good this is in agreement with the observations of Matthews and Van Dijk, (2004); and Koshy et al., (2003). Muscle relaxation may count for usage of diazepam with ketamine, since diazepam is reported to be a good muscle relaxant (Hall et al., 2001).

Different reflexes were monitored during the course of anaesthesia in this study in attempt to measure reliability of these reflexes in monitoring anaesthesia, but although some of these reflexes were used in the literature to monitor anaesthesia: palpebral and pedal reflex (Williams and Wyatt, 2007), palpebral, corneal, eyelid reflex (Tamiisto et al., 1981) spinal reflex were used as a sign of recovery.

In this study, we found that there was irregularities in the corneal and palpebral reflex and eyes remain opened and this is partially supported by Thurmon et al., (1996) who reported that eyes usually remained open with pupil dilated in animal anaesthetized with ketamine, while pedal and anal reflexs were not affected in the three protocols used, this result is supported by Susan and Donald, (2003) who stated that ketamine does not abolish pedal, photic and corneal reflex. The presence of anal reflex during anaesthesia in this study is in the same line with (Bakheit, 2010) who found that ketamine/diazepam, ketamine /xylazine and ketamine/ medetomedine protocols did not affect the anal reflex in goats.
In this study we found that the quality of recovery in all protocols used are acceptable but reduced with increasing the dose of diazepam, this might be attributed to muscle relaxation, our finding is partially supported by other findings (Matthews et al., 1991) who stated that, anaesthesia with combination of tiletamine/zolazepam in horse following 1.1 mg/kg xylazine, recoveries are difficult if the muscle relaxant effects are more prolonged than unconsciousness; the horse has difficulty in standing, or is weak and wobbly once on its feet and diazepam do not improve recovery and may lead to delay in recovery.

The lack of severe cardio-respiratory changes and absence of spinal reflex and any skeletal muscle activity during anaesthesia were indicative of acceptable clinical levels of anaesthesia in all three protocols used.

**Conclusion**

Here in this study it is to conclude that all anaesthetic protocols used were proved to be safe for the use in local donkeys in Sudan under field conditions. Increasing the dose of diazepam in the tested protocols resulted in non significant prolongation of anaesthetic phase, while the lateral recumbancy increased significantly in the high dose (0.3mg/kg diazepam) and may contribute to the delay in recovery. Although the three protocols used in the donkeys resulted in significant decrease in the respiratory rate, however no profound effect was noticed in the cardiopulmonary parameters.

**References**


anaesthetic protocols using lidocaine or medetomidine in horses. Veterinary Anaesthesia and Analgesia, 34 (4): 257-268


تقويم بعض البروتوكولات المستخدمة لحداث التخدير في الحمر السودان

preneur Ahmed (1)، محمد أحمد حسن قرشي (2) و هشام اسماعيل سري (3)

1. كلية العلوم البيطرية، جامعة نيا
2. جامعة السودان المفتوحة
3. كلية الطب البيطرية، جامعة السودان للعلوم والتكنولوجيا

المستخلص

هدفت هذه الدراسة إلى إستقصاء البروتوكولات المناسبة التي يمكن تكوينها عند استخدام عقار الكيتامين كمحذف للتخدير مع الديازيبام والزيلازين كعلاج تمهيدي لإحداث تخدير وريدي في الحمر بصورة آمنة ومرضية عند الاستخدام في الظروف الحقلية. في هذه الدراسة تم اختيار ثلاثة من البروتوكولات تتكون من عقار الكيتامين (3 مجم/كم) والديازيبام (1.5 مجم/كم) والزيلازين (0.1 مجم/كم). أجريت هذه الدراسة على ما مجموعه 18 من الحمر، من الذكور و 12 من الذكور، قسمت هذه الحيوانات بصورة عشوائية إلى ثلاثة مجموعات، و خضعت كل مجموعة لواحد من البروتوكولات السابقة. القياسات التي أجريت قبل وبعد التخدير بانتظام كل 10 دقائق هي: معدل التنفس، معدل ضربات القلب ودرجة حرارة الجسم. أما القياسات التي أجريت خلال فترة التخدير فقط هي: زمن إحداث التخدير، فترة التخدير، فترة الإستمقاء الجانب، فترة الاستمقاء القصري، فترة الوقوف، زمن الافاقية الكلية والمنعكسات العصبية. كما وتبت ملاحظة نوعية أحداث التخدير، ارتخاء العضلات و نوعية الافاقية. أحدث البروتوكولات الثلاثة درجات مقربة من إحداث التخدير وارتخاء العضلات و الافاقية. الدراسة أظهرت نقص ذو دلالة معنوية في معدل التنفس بعد حقن البروتوكول الأول خلال التخدير عدا الفترة 2، والثاني خلال كل فترة التخدير أما الثالث أظهر انخفاض معنوي بعد 10 و 20 دقيقة من أحداث التخدير. معدل ضربات القلب أظهرت زيادة معنوية في الدقيقة 50 بعد حقن البروتوكول الأول فقط. درج الحرارة لم تظهر أي تغيير بعد الحقن في كل البروتوكولات. كل البروتوكولات التي تم استخدامها أدت إلى إحداث التخدير، ارتخاء العضلات و زمن الافاقية الكلية بصورة مرضية و تخلص هذه الدراسة إلى أن هذه البروتوكولات آمنة بما يكفي لاستخدامها في تخدير الحمر السودانية تحت الظروف الحقلية.