Evaluation of Diazepam-Xylazine-Ketamine Anaesthesia on Physiological, Haematological and Serum Biochemistry Value of Sheep (Study in Gondar Town)

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Abstract: The study was conducted from November 2018 to January 2019 in 12 sheep, with randomly divided into two equal groups with the objective of assessing the anaesthetic efficacy and complications of xylazine-ketamine anaesthetic and the adjunct action of diazepam in xylazine-ketamine anaesthesia. There was no statistically significant difference (P<0.05) in induction time and time for unassisted standing between both groups. Salivation, ruminal tympany, urination and defecation were noted in both groups. There was a statistically significant increase (p<0.05) in hematological parameters at 2 and 24h after recovery compared to baseline value in both groups except TEC and lymphocyte no statistically significant decreased (p<0.05) during anaesthesia, 2h and 5 days after recovery. Serum biochemistry revealed no statistically significant difference (p<0.05) within groups and between two groups but blood glucose statistically significant increase during anaesthesia 2h and 24h after recovery compared to baseline value. Both anaesthetic drug combinations were safe for general anaesthesia of sheep since they did not mach cause any significant cardiopulmonary, kidney and liver disorders. However, inclusion of diazepam in xylazine-ketamine anaesthetic favored prolonged duration of anesthesia, reduced apneustic breathing, adequate jaw muscle relaxation and quick recovery from anaesthesia. Hence xylazine-diazepam-ketamine ideal drug combination for general anaesthesia in sheep.

Keywords: Anaesthesia, Diazepam, Gondar, Ketamine, Intravenous, Sheep & Xylazine.
1. INTRODUCTION

Most of the drugs lacked one or more of the properties of a general anaesthetic namely: hypnosis, analgesia or muscle relaxation. This had presented frequent attempts to improve the overall quality of anaesthesia by the combination of two or more drugs [1].

Xylazine inhibited reticuloruminial motility and so rumen atony and bloat could occur. Therefore it was advisable to fast the animal for 24 hours to reduce the risk of regurgitation and acute respiratory depression. Tolazoline and yohimbine reverse bradycardia and tachypnea noticed after xylazine administration. In sheep and goats, xylazine at a dose rate higher than 0.1 mg/kg could prove fatal. Lower doses of could be used intravenously while higher doses should be preferably used intramuscularly [1].

Diazepam, a benzodiazepine derivative had potent tranquillizing, calming, muscle relaxing and anticonvulsant effect. It was used as a preanesthetic to relieve skeletal muscle spasm and as an anticonvulsant. Diazepam was indicated in animals with a history of seizure disorders. It was frequently administered prior to ketamine hydrochloride to prevent seizures and muscle hypertonus. Diazepam acts on specific benzodiazepine receptor sites located on postsynaptic nerve endings located within the CNS. The anxiolytic and skeletal muscle relaxing effects were a result of increased availability of inhibitory neurotransmitter glycine. Sedation and anticonvulsant activity were mediated by GABA [2].

Ethiopia has a large number of sheep populations, but there is not much anaesthetic work which has been carried out on these animals. The management of surgical disease in sheep necessitates development of a safe and reliable anaesthetic protocol for clinical and field use. Hence, the present study was undertaken with the following objectives:

a. To evaluate the effect of xylazine–ketamine anaesthetic combination on physiological, haematological and biochemical changes during general anaesthesia in sheep.

b. To evaluate the efficacy of the adjunct action of diazepam in xylazine - ketamine anaesthetic combination on physiological, haematological and biochemical changes during general anaesthesia in sheep.

2. MATERIALS AND METHODS

2.1 Experimental Design

2.1.1 Study area

The study was conducted at University of Gondar Veterinary clinic, Gondar, Ethiopia for a period from November 2018 to January 2019 on evaluation of diazepam as an adjunct to xylazine - ketamine anaesthesia in sheep. Gondar town is located 738 km away north of Addis Ababa, the capital city of Ethiopia.

2.1.2 Selection of Animals

The experimental animals were 12 male apparently healthy sheep, animals were randomly divided into two groups viz., group I and II comprising of six animals each. The details of age and body weight of sheep selected for the study were presented in table 1.

<table>
<thead>
<tr>
<th>Animal No</th>
<th>Mean</th>
<th>Group I</th>
<th>Group II</th>
<th>Group I</th>
<th>Group II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2.70</td>
<td>2.45</td>
<td>25.16</td>
<td>24.33</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Age and body weight of the sheep selected for the study.
2.1.3 Anaesthetic Management and Preparation of Animals

All the animals were kept off feed and water for 12 and 6 hours respectively before anaesthesia. Body weight (BW) was measured using a weighting balance. Thorough physical examination was also performed. The sheep were positioned in sternal recumbency, the jugular areas were shaved and aseptic preparation with povidone iodine which done for collection of blood from jugular vein for haematological and biochemical tests and also for the administration of anaesthetic drug (Plate 1). All trials were carried out in the absence of any manipulative or surgical procedures. The anaesthetized sheep were then placed on left lateral recumbency their heads were supported on with bag so as to permit free drainage of saliva (plate 4).

2.1.4 Anaesthetic Protocol

The intended anaesthetic procedure was carried out as per the following protocol.

Table 2. Anaesthetic protocol employed

<table>
<thead>
<tr>
<th>Group</th>
<th>No of Sheep</th>
<th>Anaesthesia</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>6</td>
<td>Xylazine (0.1 mg/kg BW) and Ketamine (5.0 mg/kg BW) both drugs loading in the same syringe used as a single i.v injection. Maintenance of anaesthesia was done with incremental injection of ketamine I.V till effect in all the animals</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>Xylazine (0.1 mg/kg BW) Diazepam (0.5 mg/kg BW) and Ketamine (5.0 mg/kg BW) all drugs loaded in the same syringe used as a single i.v injection. Maintenance of anaesthesia was done with incremental injection of ketamine I.V till effect in all the animals</td>
</tr>
</tbody>
</table>

2.2 Physiological Parameters

The rectal temperature, heart rate, respiratory rate and rumen motility were recorded 15 minute before anaesthesia and repeated at 10, 20 and 30 min during general anaesthesia and after recovery.

2.2.1 Rectal Temperature

Rectal temperatures in degree Celsius (°C) was measured by using a clinical thermometer which was inserted at least 1.5-2.0 cm into the rectum and kept in the position for one minute [1].

2.2.2 Heart Rate

Heart rate in beats per minute was recorded by auscultation with a stethoscope placed over the left side of chest [2].

2.2.3 Respiratory Rate

Respiratory rate (breaths/minute) was counted by visual observation of thoracoabdominal movements. Care was taken not to excite the animal before, during monitoring and after recovery [2].

2.2.4 Rumen Motility

Rumen motility rates (motility/minute) were measure by hearing the motility sound of rumen with the help of stethoscope [2].

2.3 Haematological Parameters

The haematological parameters were estimated by collecting 5 ml of venous blood from the external jugular vein and which were collected in clean, dry vials containing ethylene diamine tetraacetic acid
(EDTA) 15 min before induction of general anaesthesia for as a baseline data and was repeated at 2h, 24h
and 5 days after recovery recorded in all the animals (Plate 5). The sample thus collected was subjected to
determine of the following.

2.3.1 Haemoglobin
Haemoglobin (Hb) concentration was measured and reported as gram per decilitre (g/dL) as per
Sahli’s haemometer method described by Jackson [3]. The graduated measuring tube was filled with N/10
Hcl up to graduation mark-2 and placed in the haemometer. The blood sample was drawn up to 20 marks
in pipette; the blood was transferred into the acid in the measuring tube, rinsing the pipette by drawing the
solution into it many times. The haemoglobin was converted into acid haematin within 5-10 minutes. After
10 minutes distilled water was added drop by drop, mixing the solution with the rod. It was added slowly
till the colour matched with the standard on either side of the haemometer.

2.3.2 Packed Cell Volume
The packed cell volume (PCV) was determined by capillary of blood (microhematocrit method) [3]. The
blood was drawn into the capillary tube to approximately 75 % of their heights and one end of the tube
was sealed with wax. The capillary tube loaded on the centrifuge so that the open ends of the tube must
direct toward the centre of the centrifuge and centrifuge for 4 minutes at a speed of 13,000 rpm after
centrifuge capillary tubes set on hematocrit reader and make the reading in percent.

2.3.3 Total Erythrocyte Count
The total erythrocyte count (TEC) in millions per cubic millimetre (10^6/cu mm) was calculated by
dilution and chamber technique using Hayems solution as per the methods described by [4].

2.3.4 Total Leukocyte Count
The total leukocyte count in thousands per cubic millimetres was counted by standard dilution
technique using Thomas fluid as per the methods described by [4].

2.3.5 Differential Leukocyte Count
A drop of blood was placed on clean glass slide and a thin blood smear was made with the help of
another slide using its edge at 45° angels and stained by Gemisa stain. A total of 100 leucocytes were
counted which included the all cell number expressed in percentage [4].

2.4 Serum Biochemistry
Five millilitres of blood was collected from the external jugular vein of all the animals in both the
groups in a sterile coagulant glass tube 15 min before induction of general anaesthesia and used for
baseline data and repeated during anaesthesia and at 2h, 24h and 5 days after recovery recorded in all the
animals. The samples were allowed to clot and the serum was separated by centrifuging at 3000 rpm for 5
minutes. The serum was stored in 2.0 ml vial at 4°c and label used for estimation of the following
biochemical parameters analyze by the help of biosystem-A clinical chemistry analyzer (Thermo Scientific™ Indiko™ Clinical and Specialty Chemistry System, Germany ) (plate. 6 and 7) [5].

2.4.1 Blood Glucose
The blood glucose level was measured by ACCU-CHEK® Aviva Plus system. Insert the test strip
into the meter in the direction of the arrows. Place the meter on a flat surface. Touch the drop to the front
eedge of the yellow window of the test strip do not put blood on top of the test strip until you see flash and
the test result appears on the display in mg/dL. After a successful test, the meter turns itself off 5 seconds
after the test strip is removed (Plate 8) [6].
2.4.2 Serum Creatinine
The serum creatinine level in mg/dL was analyzed by Modified Jaffe’s method by using a commercial kit (Creatinine reagent set, IMEDICA diagnostic solutions Pvt. Ltd., India) as per manufacturer's procedure. Creatinine produces a stained complex with picrate in alkaline medium. The staining intensity is proportional to creatinine concentration [7].

2.4.3 Serum Glutamic Pyruvic Transamine
Serum Glutamic Pyruvic Transamine (SGPT) level was given in unit/100 ml was analyzed modified International Federation of Clinical Chemistry (IFCC) method by using a commercial kit as per manufacturer procedure [7].

2.4.4 Serum Glutamic Oxaloacetic Transaminase (SGOT)
Serum Glutamic Oxaloacetic Transaminase (SGOT) level were given in unit/100ml was analyzed modified IFCC method by using a commercial kit as per manufacturer procedure [7].

2.5 Statistical Analysis
Results were expressed as mean ± SD. Differences in physiological, haematological and serum biochemical parameter in the two groups were analyzed using statistical tests. A paired sample t-test was used to compare the mean at different time intervals with their baseline values of physiological haematological and serum biochemical parameters within the group. Repeated measures ANOVA and one way ANOVA was used to compare the alteration of physiological, haematological, serum biochemical parameter, between two groups. Analyses were performed using commercial statistical software (SPSS, ver.16.0 demo, Chicago, IL). Statistical significance was considered if p value was <0.05.

3. RESULTS
3.1 Physiological Parameters
The rectal temperature, heart rate, respiratory rate and rumen motility were recorded 15 min before induction of anaesthesia used as baseline value, 10, 20 and 30 min during anaesthesia and after recovery in group I and II are presented in table 5.

3.1.1 Rectal Temperature
The mean (±SD) rectal temperature (ºC) recorded 15 min before induction of anaesthesia, 10, 20 and 30 min during anaesthesia and after recovery are shown in table 5. There was a statistically significant (P<0.05) decrease in the rectal temperature at 20 and 30 min of during anaesthesia when compared to baseline value and return to baseline value after recovery in group I and II. There was not a statistically significant (P>0.05) difference between group I and group II animals.

3.1.2 Heart Rate
The mean (±SD) heart rate per min recorded 15 min before induction of anaesthesia 10, 20 and 30 min during anaesthesia and after recovery are shown in table 5. There was a statistically significant decrease (p < 0.05) in heart rate at 10, 20 and 30 min during anaesthesia in both groups compare to baseline value and return to baseline value after recovery. There was not a statistically significant difference (p >0.05) between group I and group II animals.

3.1.3 Respiratory Rate
The mean (±SD) respiratory rates per min recorded 15 min before induction of anaesthesia, 10, 20 and 30 min during anaesthesia and after recovery are shown in table 5. There was a statistically significantly increased (p< 0.05) in respiratory rate at 10 min during anaesthesia but significant decrease (P<0.05) at 20 and 30 min during anaesthesia in both groups when compared to baseline value and return to
baseline value after recovery. There was not a statistically significant difference (p> 0.05) between group I and II animals.

3.1.4 Rumen Motility

The mean (±SD) rumen motility per min recorded 15 min before induction of anaesthesia, 10, 20 and 30 min during anaesthesia and after recovery are shown in table 5. There was a statistically significant decrease (p < 0.05) in rumen motility at 10, 20 and 30 min during anaesthesia in both groups when compared to baseline value and return to baseline value after recovery. There was statistically significant difference (p< 0.05) between groups at 30 min during anaesthesia.

Table 3. Mean (± SD) rectal temperature, heart rate, respiratory rate and rumen motility.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Before 15 min</th>
<th>During Anaesthesia</th>
<th>After recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10 min</td>
<td>20 min</td>
<td>30 min</td>
</tr>
<tr>
<td>Rectal Temperature (°C)</td>
<td>I</td>
<td>39.35 ±0.3</td>
<td>39.32 ±0.58</td>
<td>38.78 ±0.31b</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>39.42 ±0.38</td>
<td>38.92± 0.30</td>
<td>38.42 ±0.25b</td>
</tr>
<tr>
<td>Heart rate (beat/min)</td>
<td>I</td>
<td>78.33 ± 6.97</td>
<td>69.33 ± 4.84b</td>
<td>64.00 ± 9.03b</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>80.00± 6.19</td>
<td>66.00± 7.37b</td>
<td>67.66 ±7.34b</td>
</tr>
<tr>
<td>Respiratory Rate (rate/min)</td>
<td>I</td>
<td>29.33± 3.01</td>
<td>30.66± 4.32b</td>
<td>28.00 ±7.04b</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>27.80± 4.40</td>
<td>31.20± 4.48b</td>
<td>26.80±2.99b</td>
</tr>
<tr>
<td>Rumen Motility (motility/min)</td>
<td>I</td>
<td>1.91± 0.20</td>
<td>0.54± 0.07b</td>
<td>0.45± 0.07b</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>2.16± 0.68</td>
<td>0.54± 0.08b</td>
<td>0.49± 0.09b</td>
</tr>
</tbody>
</table>

* a p< 0.05 between groups  
  b P< 0.05 within group

3.2 Haematology

The haemoglobin, packed cell volume, total erythrocyte count, total leukocyte count, and differential leucocytes count recorded 15 min before induction of anaesthesia, during anaesthesia and 2h, 24h and 5 days after recovery are presented in table 6.

3.2.1 Haemoglobin

The mean (±SD) haemoglobin in g /dL recorded 15 min before induction of anaesthesia during anaesthesia and 2h, 24h and 5 days after recovery are shown in table 6. There was a statically significantly increase (p<0.05) in haemoglobin concentration at 2h and 24h after recovery when compared to baseline data and it returned to normal values on 5th day after recovery in group I and II. There was not a statically significantly difference (P>0.05) between groups.

3.2.2 Packed Cell Volume

The mean (±SD) PCV in percent recorded 15 min before induction of anaesthesia, during anaesthesia and 2h, 24h and 5 days after recovery are shown in table 6. There was a statistically significant increase (p<0.05) in PCV values at 2h and 24h after recovery when compared to baseline data and it returned to normal values on 5th day after recovery in group I and II. There was not a statistically significantly difference (p>0.05) between groups.

3.2.3 Total Erythrocyte Count

The mean (±SD) total erythrocyte count in millions per micro litre recorded 15 min before induction of anaesthesia, during anaesthesia and 2h, 24h and 5 days after recovery are shown in table 6. There was a non statistically significant increased in total erythrocyte count at during anaesthesia, 2h, 24h after recovery when compared to baseline data and it returned to normal values on 5th day after recovery in group I and II. There was a statistically significantly difference (P<0.05) in total erythrocyte count between groups.
3.2.4 Total leukocyte count
The mean (±SD) total leukocyte count in thousands per micro litre recorded 15 min before induction of anaesthesia during anaesthesia and 2h, 24h and 5 days after recovery are shown in table 6. There was a statistically significant increase (p<0.05) in total leukocyte count at 2h and 24 h after recovery when compared to baseline values and return to baseline value at 5 days after recovery in both groups. Statistical comparison revealed no statistically significant (p>0.05) difference in total leukocyte count between groups.

3.2.5 Differential Leukocyte Count
The mean (±SD) in percentage of neutrophils, lymphocytes, monocytes and eosinophils were recorded 15 min before induction of anaesthesia, during anaesthesia and 2h, 24h and 5 days after recovery were neutrophils, lymphocytes, eosinophils and monocytes are showed in table 6. Neutrophils and monocytes were significantly (P < 0.05) elevated at 2h and 24h after recovery compared to baseline value and return to baseline value at 5 days after recovery in both groups. There was a statistically significantly (P<0.05) decrease in lymphocyte and not statistically significant decrease in eosinophils during anaesthesia, 2h and 24h after recovery compared to baseline value and return to baseline value at 5 days after recovery in group I and II. There was a statistically significantly (P<0.05) difference in neutrophils during anaesthesia and 2h after recovery and in lymphocyte percent during anaesthesia between group I and II.

Table 4. Mean (± SD) haemoglobin, packed cell volume, total erythrocyte, total leukocyte count, differential leucocytes count.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>15 min before induction</th>
<th>During anaesthesia</th>
<th>2 h</th>
<th>24 h</th>
<th>5 day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (g/dL)</td>
<td>I</td>
<td>8.86 ± 0.61</td>
<td>8.83 ± 0.75</td>
<td>8.81 ± 0.48&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.38 ± 0.39&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.80 ± 0.77</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>8.33 ± 0.51</td>
<td>8.33 ± 0.75</td>
<td>9.33 ± 0.82&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.38 ± 0.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.67 ± 0.68</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>I</td>
<td>26.67 ± 2.58</td>
<td>27.08 ± 4.00</td>
<td>28.75 ± 2.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>28.33 ± 2.58&lt;sup&gt;b&lt;/sup&gt;</td>
<td>27.83 ± 1.08</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>26.65 ± 4.98</td>
<td>27.17 ± 3.19</td>
<td>30.00 ± 4.73&lt;sup&gt;b&lt;/sup&gt;</td>
<td>27.91 ± 2.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25.83 ± 3.06</td>
</tr>
<tr>
<td>TEC (10&lt;sup&gt;6&lt;/sup&gt;/μL)</td>
<td>I</td>
<td>10.97 ± 1.50</td>
<td>11.28 ± 0.83</td>
<td>11.29 ± 0.80</td>
<td>11.34 ± 0.46</td>
<td>10.86 ± 0.69</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>11.08 ± 0.44</td>
<td>11.24 ± 0.36</td>
<td>11.49 ± 2.24</td>
<td>11.51 ± 0.56</td>
<td>11.18 ± 0.24</td>
</tr>
<tr>
<td>TLC (10&lt;sup&gt;3&lt;/sup&gt;/μL)</td>
<td>I</td>
<td>10.95 ± 2.14</td>
<td>10.42 ± 3.11</td>
<td>11.59 ± 3.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.82 ± 2.76&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.46 ± 1.18</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>8.66 ± 1.78</td>
<td>9.62 ± 2.54</td>
<td>12.14 ± 2.37&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.55 ± 2.43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.74 ± 0.51</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>I</td>
<td>34.17 ± 2.92</td>
<td>38.50 ± 2.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>45.83 ± 2.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>45.33 ± 1.86&lt;sup&gt;b&lt;/sup&gt;</td>
<td>35.00 ± 2.00</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>33.16 ± 4.53</td>
<td>35.50 ± 4.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48.33 ± 1.75&lt;sup&gt;b&lt;/sup&gt;</td>
<td>44.33 ± 4.37&lt;sup&gt;b&lt;/sup&gt;</td>
<td>35.00 ± 2.00</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>I</td>
<td>53.50 ± 2.07</td>
<td>50.00 ± 1.27&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40.00 ± 2.96&lt;sup&gt;b&lt;/sup&gt;</td>
<td>42.67 ± 3.44&lt;sup&gt;b&lt;/sup&gt;</td>
<td>53.50 ± 1.38</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>56.00 ± 5.14</td>
<td>55.17 ± 5.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>40.67 ± 2.73&lt;sup&gt;b&lt;/sup&gt;</td>
<td>45.17 ± 3.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>54.50 ± 2.95</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>I</td>
<td>4.50 ± 0.83</td>
<td>5.33 ± 1.03</td>
<td>9.17 ± 2.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.00 ± 1.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.67 ± 0.82&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>4.00 ± 1.09</td>
<td>4.67 ± 0.81</td>
<td>6.83 ± 3.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.50 ± 2.34&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.33 ± 1.21&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>I</td>
<td>7.83 ± 1.48</td>
<td>6.17 ± 1.17</td>
<td>5.17 ± 0.75&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.00 ± 2.00</td>
<td>7.00 ± 1.67</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>6.83 ± 1.72</td>
<td>6.5 ± 1.51</td>
<td>4.16 ± 1.60</td>
<td>5.00 ± 1.27</td>
<td>6.86 ± 1.48</td>
</tr>
</tbody>
</table>

3.3 Serum Biochemistry
The mean (± SD) blood glucose serum creatinine, SGPT and SGOT was recorded 15 min before induction of anaesthesia, during anaesthesia, 2 h, 24 h and 5 days after recovery are presented in table 7.

3.3.1 Serum Glucose
The mean (±SD) blood glucose in gm/dL recorded 15 min before induction of anaesthesia, during anaesthesia and 2 h, 24 h and 5 days after recovery are showed in table 7. There was a statistically
significant elevate (p<0.05) at during anaesthesia and 2 h after recovery when compared to baseline data and it non statistically significant decreased (p < 0.05) at 24 h after recovery and 5 day after recovery in group I and II. There was significant (p < 0.05) difference at 24 h after recovery. between group I and II.

### 3.3.2 Serum Creatinine

The mean (±SD) creatinine in mg/dL recorded at 15 min before induction of anaesthesia, during anaesthesia and repeated at 2h, 24h and 5 days after recovery are shown in table 7. Statistical analysis did not reveal statistically significant (p > 0.05) difference in creatinine level at different stages of anaesthesia within both groups and between groups.

### 3.3.3 Serum Glutamic Pyravic Transaminase (SGPT)

The mean (±SD) SGPT level in unit/100 ml recorded at15 minute before induction of anaesthesia, during anaesthesia and 2h, 24h and 5 days after recovery are shown in table 7. There was not a statistically significant (p >0.05) difference with in both groups at different stages of anaesthesia but statistically significant difference at 2 h after recovery between groups.

### 3.3.4 Serum Glutamic Oxaloacetic Transaminase (SGOT)

The mean (±SD) SGOT level in unit/100 ml recorded at15 minute before induction of anaesthesia, during anaesthesia and 2h, 24h and 5 days after recovery are shown in table 7. There was no statistically significant (p >0.05) difference with in both groups at different stages of anaesthesia but statistically significant difference (p<0.05) at 24 h after recovery in between groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Before 15 min</th>
<th>During anesthesia</th>
<th>After recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2h</td>
<td>24h</td>
<td>5 day</td>
</tr>
<tr>
<td>Blood Glucose (gm/dL)</td>
<td>I</td>
<td>95.16 ± 20.24</td>
<td>117.17± 20.12(^b)</td>
<td>139.33 ± 22.33(^b)</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>92.42 ± 15.09</td>
<td>124.5 ± 30.01(^b)</td>
<td>150.83 ± 37.02(^b)</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>I</td>
<td>1.20 ± 0.28</td>
<td>1.36 ± 0.24</td>
<td>1.41 ± 0.28</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>0.93 ± 0.47</td>
<td>1.11 ± 0.42</td>
<td>1.16 ± 0.28</td>
</tr>
<tr>
<td>SGPT (U/100ml)</td>
<td>I</td>
<td>196.15 ± 9.48</td>
<td>182.9 ± 11.35</td>
<td>188.48 ± 11.07(_a)</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>174.76 ± 29.98</td>
<td>170.7 ± 28.98</td>
<td>167.6 ± 26.2(_a)</td>
</tr>
<tr>
<td>SGOT (U/100ml)</td>
<td>I</td>
<td>47.33 ±16.91</td>
<td>40.81 ± 16.47</td>
<td>41.40 ± 10.82</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>52.53± 10.28</td>
<td>45.06 ± 8.97</td>
<td>47.97 ± 7.24</td>
</tr>
</tbody>
</table>

\(^a\) p< 0.05 between groups \(^b\) P< 0.05 within group

### 4. DISCUSSION

This anaesthetic study conducted in two groups of twelve sheep selected with similar age and body weight was suitable to obtain more consistent data and facilitated statistical comparison. The uniformity in age and body weight of the animals among the groups helped to study the response associated with the anaesthetic protocol more precisely. Perusal of literature revealed not much of detailed study on the influence of age and body weight of sheep on the outcome of the anaesthetic protocol.

The withholding feed and water for 12 and 6 h, respectively prior to induction of anaesthesia was found suitable and prevented potential complications associated with regurgitation of ruminal contents.
This could be attributed to the reduction of ruminal contents. This concurs with the recommendations of [8, 9].

There was not statistically significant (p >0.05) difference in time taken for standing between groups. This may be due to the IV route of administration of anaesthetics which favours rapid drug biotransformation [1].

The changes recorded in rectal temperature, heart rate, respiratory rate and rumen motility during the study could be attributed to the anaesthetic protocols employed. On the basis of literature, both xylazine -ketamine and xylazine-diazepam-ketamine may produce hypothermia [10].

In the present study statistically significant (p <0.05) reduction of rectal temperature was observed at 20 and 30 min during anaesthesia in group I and II animals. It could be attributed to the administration of diazepam, xylazine and ketamine which leads to CNS depression and reduction of muscular activity, decrease in metabolic rate and α2-Agonists effects. The prolonged depression of thermoregulation with decline in body temperature observed in this study is in accordance with the findings of [11, 12].

The results of this study showed that there was a decrease in heart rate at 10, 20 and 30 min during anaesthesia in both group. The cause of the slight fall in heart rate at the 10 minute intervals relative to base line values may be due to CNS depression, similarly reduction in heart rate using xylazine-ketamine combination was reported by [13]. The xylazine-ketamine-diazepam drug combination was also reported to reduce heart rate [14]. This might be due to the major side effects of α2- adrenergereceptor agonists on the cardiovascular system that may have decreased the heart rate in these anaesthetic regimens [15]. Although ketamine may increase the heart rate by the increased sympathetic activity and decreased vagal tone, xylazine overrides these effects by excitatory carotid bar receptor reflex induced by hypotension and decreased sympathetic and increased vagal activity [15].

Diazepam produced hypotension, bradycardia and cardiac arrhythmias in group II animals. These effects might be due to propylene glycol, which is a cardiovascular depressant. This observation of reduced heart rate concurs with the observation of Gled [16, 17, 14]. Similar studies using xylazine- ketamine and xylazine-diazepam -ketamine combination have also been reported to lower heart rate following administration [18, 13, 14, 1, 19].

There was statistically significant decrease (p<0.05) in respiratory rate at 20 and 30 min during anaesthesia in group I and group II animals, which may be attributed to depression of respiratory centre in CNS. The apneustic pattern of breathing observed in this study may be due to ketamine hydrochloride and respiratory depression due to administration of benzodiazepines, direct depression of central respiratory drive and simultaneous depression of respiratory muscle efficiency. Similar observation have been reported by others workers [18, 20, 12, 21, 19].

There was a statistically significant decrease (P< 0.05) in rumen motility at 10, 20 and 30 min during anaesthesia in both groups compared to baseline value and return to baseline value after recovery. Similar observations were reported in previous studies in ruminants using xylazine and diazepam. There was a statistically significant reduction (p < 0.05) in rumen motility in sheep and goats following induction of anaesthesia using xylazine–ketamine–diazepam [14,] and xylazine anaesthesia [19, 2002]. The reduction in rumen motility after xylazine administration has been attributed to sympathetic discharge and reduced release of norepinephrine and ultimately reduced the rumen motility [22, 14].

There was a statistically significant increase (p < 0.05) in haemoglobin concentration during this study in group I and II. These results are in accordance with observations made by Symonds [23] in cattle, Custer [24] in camels and buffaloes, Fani [25] in dogs and Mohammed [19] in rats. The increase in haemoglobin concentration might be due to sequestration of blood cells from the spleen and lungs during anaesthesia [14].

There was a statistically significant (P< 0.05) elevated of PCV values at 2h and 24h after recovery in both groups when compared to baseline value. The increase in PCV values could be due to mild dehydration, stress via increased release of catecholamine, which can result in contraction of spleen and consequently increase PCV. This finding concurs with Carrol, and Mohammed [26, 19]. The increase in PCV and Hb will enhance the ability of the blood to deliver oxygen and drugs to the tissues. However the
elevated PCV is of concern to the anaesthetist because of hemoconcentration and increased blood viscosity [19].

There was a statistically significant increase (p<0.05) in total leukocyte count in 2h and 24h after recovery in both groups. The increase in total leukocyte counts observed in the present study may be due to typical of corticosteroid induced stress response, tissue damage and handling. This finding concurs with Carrol [26] and Ismail [14]. However it differs with findings of Pawde [27] and Mohammed [19] who opined that decrease TLC might be due to pooling of circulating blood cells in the spleen and other reservoirs secondary to decreased sympathetic activity.

In the presented study the percentage of neutrophils and monocytes was recorded to statistically significant increase (p < 0.05) but statistically significant (p < 0.05) decrease in lymphocyte and eosinophils at 2 h and 24 h after recovery in group I and II. It has been reported that differential leukocyte counts might be altered by stress events, such as capture and handling and corticosteroid-induced changes which included a mature neutrophilia, lymphopenia, and eosinopenia [8, 14].

There is a statistically significant (p < 0.05) increases in blood glucose levels during anaesthesia, and 2h and 24h after recovery when compared to baseline value and return to baseline value 5 days after recovery. This could be attributed to the increased in adrenaline and/or corticosteroids secretion. This concurs with the findings of Ismail [14] who reported that glucose level was significantly elevated at 2 h after recovery from xylazine-ketamine-diazepam anaesthesia in sheep and goats. Several other workers have also reported a significant increase in glucose values after xylazine/ketamine administration in deer, sheep and dogs, [14, 19]. The elevation of blood glucose may also be due to hyperglycaemic effects of xylazine in different species [28, 19]. The hyperglycaemia might also be due to the result of α2- adrenergic receptor inhibition of insulin, a stimulation of a2-adrenoreceptors in the pancreatic β cells and increased glucose production in the liver [29].

There is a statistically significant (p < 0.05) difference in serum creatinine at different time intervals of anaesthesia in both the groups implying no nephrotoxicity associated with xylazine-ketamine and xylazine- diazepam-ketamine anaesthesia. The resulting indicated that the anaesthetic drugs used in this trial did not cause any impairment in glomerular filtration or renal blood flow. The observations of the present study concur with [14, 19].

In the present study the quality of anaesthetic recovery was assessed and graded as smooth and rough. The anaesthetic recovery was smooth in four and five animals in group I and II, respectively. Complication like regurgitation, aspiration and mortality were not observed in the present study which may probably be due to retention of oropharyngeal reflexes in xylazine-ketamine anaesthesia and also the absence of increased intra ruminal pressure [30, 19].

5. REFERENCE
30. Thurmon, J.C., Tranquilli, W.J. and Benson, G.J., 1996. Lumb and Jones’ Veterinary Anaesthesia. 3rd edn., Williams and Wilkins, Baltimore, USA.