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Effects of diazepam anaesthesia on complete blood count in West African dwarf goat

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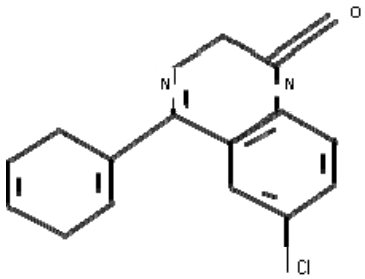
Abstract
To examine the effects of diazepam anaesthesia on the complete blood count in six (6) adult healthy West African Dwarf goat weighing 13kg to 16kg were used. Anaesthesia was induced using 0.05ml/kg diazepam intravenous injection, complete blood counts analysis was performed before anaesthesia (T.0), 2 hours after recovery, 24 hours after recovery, 5 days after recovery and 7 days later. Hematological analysis indicated that there was significant decrease in neutrophil percentages ($p \geq 0.05$) at 2 hrs, and increased significantly at 24 hours, 5 days and 7 days of recovery. There was a mild lymphopenia at 2 hours after recovery, and lymphocytosis ($p \geq 0.05$) significantly occur at 24 hours, 5 days and 7 days of recovery. There was mild decreased in monocyte percentages ($p \leq 0.05$) at 2 hours after recovery, while increased significantly at 24 hours of recovery and decrease at 5 days and 7 days of recovery. The percentages of basophil increased at 2 hours of recovery, and decrease at 24 hours of recovery, while at 5 days of recovery it increase and decrease at 7 days of recovery. The percentages of eosinophils decreased significantly at 2 hours, after recovery and significantly increase of eosinophils at 24 hours, 5 days and 7 days after recovery. The total red blood cell count decreased significantly at 2 hours after recovery, and increase at 24 hours after recovery, and it decrease significantly at 5 days after recovery, and significantly increased at 7 days after recovery. It is therefore concluded that diazepam can be used to induced short term anaesthesia in male Adult goat (West African Dwarf goat) with minimum effects on complete blood counts.

Keywords: Diazepam, West African Dwarf Goat, Complete blood counts, Intravenous injection.

1. Introduction
Diazepam is a benzodiazepine tranquilizer. Drugs in this class act indirectly on the brain and are central nervous system (CNS) depressants. Although the precise mechanism of action is not fully known, specific benzodiazepine receptors have been found in the brain and other tissues including the heart, kidney, liver and lungs.
Diazepam is used clinically for sedation, to diminish anxiety or modify behaviour, as a muscle relaxant, an anticonvulsant and in some species as an appetite-stimulant. It may be administered intra-venously, intra-muscularly, orally and rectally via suppository or anemia. Diazepam is metabolized by the liver to nordiazepam and other active metabolites. They are excreted in the urine. Diazepam can also be used a sedative either alone or in combination with other drugs. It is considered a relatively safe pre-operative sedative for patients with underlying cardiac or metabolic disease because it causes minimal cardiopulmonary-side effects and provides good muscle relaxation. It may be used in combination with ketamine for induction of anaethesia. (Mouallen, 1988)



Systematic (IUPAC) Name
7-Chloro-1, 3-dihydro-
1-Methyl-5-phyeny
1, 4-benzodiazepine-2(3H) one

2. Materials and methods

2.1 Study Area

Mokwa is the headquarter of Mokwa Local government area of Niger State, Nigeria is located at latitude 8^0 00 to 9^0 18N and longitude $4^0$00 to 5^0 04E (Isah,2008).It has annual rainfall of between 40mm to 1400mm and a mean temperature of about 24 ^{0}c to 32 ^{0}c in the day and 20 ^{0}c to 24 ^{0}c at night.

The soil is sandy loamy (Anonymous, 2006) and vegetation is typical guinea savannah.

2.2 Materials

- Hamacytometer
- Cover glass
- Pipette
- Counting chamber
- Light microscope
- Hand digit counter
- Normal saline
- Fitter paper.

2.3 Drug-Diazpam (0.1-0.2mg/kg I.V)

Experimental Animals

The experimental animals were six (6) adult healthy goats, their body weight was measured. The goat was kept in the livestock farm and feed with grasses and legumes. The goats were allowed to acclimatize for one week before the experiments commenced. The pen was properly clean before used and the goats were placed on a good management system before the commencement of experiments.

2.4 Method of obtaining blood sample from goat

Six (6) goats were used to obtained 30 blood samples. Before administration of Diazepam blood was collected and at 2hours, 24hours, 5 days and 7 days of recovery the blood samples were also collected.

The following steps were taken collecting the blood samples from goat.

- The hair over the vein was shipped
- The skin was clean with methylated spirit.
- The needle was inserted in to jugular furrows with the point forwards the animals head to obtain 5ml of blood sample i.e from goat.
- The blood sample was collected directly in to ethlenetera-acetic acid (EDTA) bottle.
- The blood was ejected from the spring with great care to avoid haemolysis of red blood cell.
- The blood sample was preserved in a good vacuum flask that can lasted than 8-12 hours.

2.5 Method of Red Blood Cell Counts

- The hemacytometer and cover glass was clean
- 2 – 3 inversion blood samples was mixed.
- The pipette was wipe out without removing and blood from the bore of the pipette.
- The reservoir was squeeze with index finger over the opening of the over flow chamber.
- The finger was remove from the over flow chamber and pressure was release at approximately the same time.
- The pipette was converted to a dropper assembly.
- The both side of hemacytometer was filled.
- The hemacytometer was place on the microscope, by using x10 objective lens, and it was insured that the objective lens does not tough the hemacytometer.
- The microscope condense was lowered and course focus knob was use to bring the cells in to rough focus to locate the proper squares to be countered.
- The fine focus knob was use to bring the cells in to sharp.
- The whole cells within the cover and counted small squares of the large counter square was counted, and those cells touching the upper and left lines was not be counted.
- The counts cells was total from the two side and formula provided i.e number of cells count x 10,000 per cubic mm.
- The hemacytometer and cover glass was clean with distilled water and was dried with laboratory tissue.(Lewis 1976)

2.6 Method of white blood cell counts

- The blood collected from six (6) goats was mixed with a weak acid solution that lyses nonucleated red blood cells.
- The blood sample was thoroughly mixed and was introduced to a counting chamber where the white blood cells (leukocytes) in a diluted form is counted.
- Two percent acetic acid and 2ml of glacial acetic acid was added to 100ml volumetric flask.
- Mark dilute was use by using distilled water.
- Pipette was used to draw well-mixed venous blood to the 0.5 mark in a white blood cell diluting pipette. The blood column must be free of air bubbles.
- The access blood was wipe outside of the pipette to avoid transfer of cells to the diluting fluid.
- The care was taking in order not to touch the tip of the pipette with the gauze.
- The diluting fluid was immediately draw to the “11” mark while rotating the pipette between thumb and forefinger to mix the sample and diluents.
- The pipette was hold upright to prevent air bubbles in the bulb.
- The contents of the pipette was mixed for 3 – 5 minutes to ensure even distribution of cells.
- The mixture was allow to flow under the cover glass until the chamber is completely charged.
- The opposite chamber of the hemacytometer was fill.
- The cell was allow to settle for about 3 minutes under low power magnification and the light was reduced, focus on the ruled area and even distribution of cells was observed.
- The white cells was count in four 15qmm corner area corresponding to those marked A, B, C and D figure 5 – 1 each of the two chambers.
- All the white cells lying within the square and those touching the upper and right-hand center lines was counted.
- The white cells that touch the left-hand and bottom line was not counted.
- In each of the four chamber area counting was conducted as indicated by the “Snake-like” line in figure 5 – 1.
- A variation of more than 10 – 20 cells between any of the four areas counted between side of the hemacytometer indicate uneven distribution and required that the procedure was repeated.(Lewis 1976)

2.7 Method of Analysis

The data obtained were summarized as means and analysis of variance (ANOVA).

3. Result and Discussion

Results

The percentage of lymphocyte following the intravenous administration of diazepam in Goat.

Goat	Control (%)	2hrs (%)	24hrs (%)	5days (%)	7dyas (%)	Total	Means
A	36	31	38	39	37	181	36.20
B	40	36	46	49	42	213	42.60
C	41	38	45	46	42	212	42.40
D	35	30	37	39	37	178	35.60
E	45	41	49	43	46	224	44.80
F	35	32	38	40	37	182	91.00
Total	**232**	**208**	**253**	**256**	**241**	**1190**	
Means	**38.67**	**34.67**	**42.17**	**42.67**	**40.17**		

The percentage of lymphocyte following the intravenous administration of diazepam in Goat.

Goat	Control (%)	2hrs (%)	24hrs (%)	5days (%)	7dyas (%)	Total	Means
A	60	54	66	68	62	310	62.00
B	55	50	57	58	56	276	55.20
C	50	49	53	56	52	260	52.00
D	70	67	75	78	72	362	72.40
E	60	55	65	68	61	309	61.80
F	55	51	59	61	57	283	56.60
Total	**350**	**326**	**375**	**389**	**360**	**1800**	
Means	**58.33**	**54.33**	**62.50**	**64.83**	**60.00**		

The percentage of monocyte following the intravenous administration of diazepam in Goat.

Goat	Control (%)	2hrs (%)	24hrs (%)	5days (%)	7dyas (%)	Total	Means
A	2	2	3	2	3	12	2.40
B	1	0	2	2	2	7	1.40
C	4	2	5	3	1	15	3.00
D	0	0	1	1	2	4	0.80
E	0	1	2	3	2	8	1.60
F	4	1	0	3	5	13	2.60
Total	**11**	**6**	**13**	**14**	**14**	**59**	
Means	**1.83**	**1.00**	**2.67**	**2.33**	**2.33**		

The percentage of Eosinophils following the intravenous administration of diazepam in Goat.

Goat	Control (%)	2hrs (%)	24hrs (%)	5days (%)	7dyas (%)	Total	Means
A	4	2	3	4	5	18	3.60
B	5	3	3	5	6	22	4.40
C	3	2	2	2	5	14	2.80
D	5	3	2	5	4	19	3.80
E	2	1	3	2	3	11	2.20
F	3	2	3	3	3	14	2.80
Total	**22**	**13**	**16**	**21**	**26**	**98**	
Means	**3.67**	**2.17**	**2.67**	**3.50**	**4.33**		

The percentage of Basophis following the intravenous administration of diazepam in Goat

Goat	Control (%)	2hrs (%)	24hrs (%)	5days (%)	7dyas (%)	Total	Means
A	0	1	1	1	1	4	0.80
B	1	0	0	2	0	3	0.60
C	1	0	1	3	1	6	1.00
D	0	1	0	0	1	2	0.40
E	0	1	0	1	1	3	0.60
F	0	0	0	0	0	0	0.00
Total	**2**	**3**	**2**	**7**	**4**	**18**	
Means	**0.33**	**0.50**	**0.33**	**1.17**	**40.17**		

The percentage of White Blood Cell (WBC) ($X10^6/\mu L$) following the intravenous administration of diazepam in Goat.

Goat	Control (%)	2hrs (%)	24hrs (%)	5days (%)	7dyas (%)	Total	Means
A	197	129	132	136	199	793	158.60
B	138	157	159	162	131	747	149.40
C	160	102	100	106	163	631	126.20
D	109	196	197	198	111	811	162.20
E	200	198	199	100	204	901	180.20
F	110	105	108	111	114	548	109.60
Total	**914**	**887**	**895**	**813**	**922**	**4431**	
Means	**152.33**	**147.83**	**149.17**	**135.50**	**153.67**		

The percentage of Red Blood Cell (RBC) ($x10^6/\mu L$) following the intravenous administration of diazepam in Goat.

Goat	Control (%)	2hrs (%)	24hrs (%)	5days (%)	7dyas (%)	Total	Means
A	90	88	92	94	93	457	91.40
B	95	86	97	99	97	474	94.80
C	96	91	98	100	97	1301	260.20
D	80	78	83	85	83	409	81.80
E	97	93	99	100	99	488	97.60
F	81	77	113	117	82	470	94.00
Total	**539**	**513**	**582**	**595**	**551**	**3599**	
Means	**89.83**	**85.50**	**97.00**	**99.17**	**91.83**		

4. Discussion

4.1 Effects of Daizepam on Neutrophil

The result shown that before the administration of diazepam the volume of neutrophil is (T.0) 38.67%, at 2 hours of recovery the volume decreased to 34.67%, at 2 hours of recovery the volume increased to 42.17%, at 5 days of recovery the volume increased to 42.6%, and at 7 days of recovery the volume increased to 40.17% these means that diazepam had effect of diazepam $P \geq 0.05$ these were significant different.

4.2 Effects of Diazepam on the Lymphocyte

The result shown that before the administration of diazepam the volume of lymphocyte is (T.0) 58.33%, at 2hours of recovery the volume decreased to 54.33%, at 24 hours of recovery the volume increase to 62.5%, at 5 days of recovery the volume increased to 64.83% and at 7 days of recovery the volume decreased to 60.00%. $P \leq 0.05$ these were significant different.

4.3. Effects of Diazepam on Monocytes

The result shown that before the administration of diazepam the volume of monocyte is (T.0) 1.83%, at 2 hours of recovery the volume decreased to 1.00%, at 24 hours of recovery increased to 2.67%, at 5 days of recovery the volume decreased to 2.33% and at 7 days of recovery the volume is still at 2.33%, when compared with control value. $P \leq 0.05$ these were the significant different.

4.4 Effects of diazepam on the Eosinophil

The result show that before the administration of diazepam the volume of Eosinophil is (T.0) 3.67%, at 2 hours of recovery the volume decreased to 2.17%, at 24hours of recovery the volume increased to 2.67%, at 5 days of recovery the volume increased to 3.5% and at 7 days of recovery the volume increased to 4.33%, when compared with control value $P \geq 0.05$ that mean these were significantly different.

4.5 Effects of Diazepam on the Basophil

The result shown that before the administration of diazepam the volume of Basophils is (T.0) 0.33%, at 2 hours of recovery the volume increased to 0.50%, at 24 hours of recovery the

volume decreased to 0.33%, at 5 days of recovery the volume increased to 1.7%, and at 7 days of recovery the volume decreased to 0.67%, when compared with the control means. P<0.05 these were significant different.

4.6 Effects of Diazepam on Red Blood Cells (R.B.C) ($x10^6/\mu L$

The result shown that before the administration of diazepam the volume of Red Blood Cells (R.B.C) is (T.0) 89.83%, at 2 hours of recovery the volume decreased to 85.50%, at 24 hours of recovery the volume increased to 97.00% at 5 days of recovery the volume increased to 99.17% and at 7 days of recovery the volume decreased to 91.83%, P<0.05 mean that these were significant different.

4.7 Effects of Diazepam on White Blood Cells (W.B.C) ($x10^6/\mu L$)

The result shown that before the administration of diazepam in the volume of white blood cells is (T.0) 152.33%, at 2 hours of recovery the volume decreased to 147.83%, at 24 hours of recovery the volume increased to 149.17%, at 5 days of recovery the volume decreased to 135.50%, and at 7 days of recovery the volume increased to 153.67%. P<0.05 that means these were significant different.

5. Conclusion

Diazepam is a useful pre-anaesthetic agent, sedative short acting analgesic in animals. Its major use is found when it is combined with xylazine and ketamine to provide anaesthesia with visceral analgesia.

However, the drug (diazepam) in this case induced short term anesthesia in goat (West Africa Dwarf) with minimum effects on the complete blood counts, particularly in the leucocyte and erythrocyte counts.

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