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Effects of diazepam anesthesia on the total blood protein and blood glucose in West African dwarf goat

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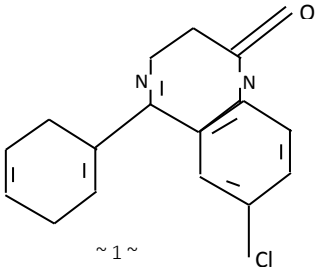
Abstract
The effects of diazepam anesthesia in total blood protein and blood glucose was determined in six adult, healthy West African dwarf goats weighing 13kg to 16kg were used. Anesthesia was induced using 0.25mg/kg diazepam intravenous injection. Total blood protein and blood glucose analysis were performed before anesthesia (To), 2 hours, 24 hours, 5 days and 7 days after recovery. Effects on clinical parameters the result indicated that there were no significant changes in hemoglobin and albumin concentrations while in glucose there was significant decreased at 2 hours and 24 hours after recovery. It is therefore concluded that diazepam as a single dose can be used to induce short-term anesthesia in goats with minimum effects on clinical parameters.

Keywords: Diazepam, Hemoglobin, Albumin, Glucose,West African Dwarf Goat

1. Introduction
Diazepam is one of the central Nervous system (CNS) depressants is a category of drug that slow normal brain function. Diazepam was the second benzodiazepine family to be invented by Dr. Deosternbach of Hoffimann-Laroche, following chlordiazepoxide (Librium, diazepam become incredibly popular, helping Roche to become a pharmaceutical industry giant.
Diazepam is mainly used to treat anxiety, insomnia, long-term treatment of epilepsy, panie attack, tetanus, stroke, spinal cord injury, stiff person syndrome and symptoms of acute alcohol withdrawal.
Benzodiazepines do not have any pain relieving properties of themselves and are generally recommended to be avoided in individual with pain. However, Benzodiazepines such as diazepam can be used for their muscle relaxant properties to alleviate pain, which is caused by muscle spasms, cause by various dystopias, including blepharospam. Also diazepam is sometimes is sometimes used intermittently for the prophylaxis of febrile seizures which occur as a result of a high fever in children and neonates under 5 years of age, and also diazepam has known as a broad spectrum of indications (most of which are off-label).
Diazepam can be administered orally, intravenously, (I.V) need to be diluted as it is painful and damaging to the veins, intramuscularly (I.M) injection (this is painful and not advised) absorption is slow, erratic and incomplete. Diazepam is highly protein bound with 96 – 99 percent of the absorbed drug being protein bound. The distribution half-life of diazepam is 2 minutes to 13 minutes and is highly lipid-soluble, and is widely distributed throughout the body after administration. It easily crosses both the blood, brain barrier and the placenta, and is excreted in to breast milk. After absorption, diazepam is distributed in to muscle and adipose tissue. There is preferential storage of diazepam in some organs including the heart. Absorption by any administered route and risk of accumulation is significantly increased in the neounte and there is clinical justification to recommend the withdrawal of diazepam during pregnancy and breast-feeding.

1.1. Chemical Structure of Diazepam



1.2 Systematic (IUPAC) name

7 – Chloro – 1, 3 dihydro
1 – meltyl – 5 – phyeny
1, 4 – benzzodiapin – 2 (3H) – one.

2. Materials and Methods

2.1 Study Area

Mokwa, headquarters' of Mokwa Local Government Area of Niger State, Nigeria is located at latitude 8º 00 to 9º 18'N and longitude 4º00 to 5004 E (Isah, 2008). It has annual rainfall of between 40mm to 1400mm and a mean temperature of about 24ºc to 32º in the day and 20ºc to 24º at right.
The soil is sandy loamy (anonymous, 2006) and vegetation is typical guinea savannah.

2.2 Materials/Equipment

The following are materials/equipment used during the experiment.

- Disinfectant (Septol)
- Mentholated Spirit
- Cotton wool
- Surgical gloves
- Stethoscope
- Clinical thermometer
- Syringe and needle
- Surgical table
- Weighing scale
- Hair scrapper or Razor blade
- Autoanalyzer
- Blood glucose meter
- Centrifuge
- Test strip
- Spectrum machine
- Water bath
- Universal bottles

2.3 Drug

Diazepam (0.1 – 0.2mg/kg 1.v)
Experimental Animals
The experimental animals were six adult, healthy, male goats. Their body weight was measured.
They were acquired from Zugruma market in Mashegu Local Government. The goats were kept in intensive housing in the College livestock farm and feed on grasses, legumes and water. The goats were allowed to acclimatize for one week before the experiments commence.

2.4 Experimental Procedure

The drug was induced or administer intravenously for each goat and 30 blood samples were collected. Before the administration of diazepam, the blood samples were collected, after 2hours, 24hours, 5days and 7days of recovery, the blood samples were also collected.
The following steps were taking in collecting blood samples:

- The hair over the vein was clipped.
- The skin was cleaned with methlated spirit
- The needle was inserted in to jugular vein with the point forward the animal head to obtained blood from goat.
- The blood sample was collected directly into universal bottle.
- The blood was ejected from the spring with great care to avoid haemolysis of red blood cells.
- The blood samples were taken to the general hospital Mokwa, for the tests immediately after ejections.

2.5 Method of Total Protein Analysis

The blood sample was placed in to machine called a centrifuge, which spins the blood to separate the liquid part of the blood (the serum) from the cells. The total protein test is done on serum.
The total protein test is faster and cheaper test that estimates the total of fractions together, this fraction can be quantitated using protein uses the biuret reagent, but other chemical methods are now available such as kjeldahl method, dye binding and refractometry.

- The tubes were labeled and arranged in order and one serve as a stander (extra).
- 1ml of reagent was added to each tube
- 25 micro liters of another reagent was pipette into a stander tube
- 25 micro liters of blood serum was pipette into each tube prepared.
- The tubes were placed into water bath and the tubes were closed with cotton wool for about 10 minutes.
- Another tube was prepared with reagent and water to black the spetrol-photometer machine and was adjust it to zero level.
- Wave length of which total protein is determined is 540nm.
- Each sample was poured on curvet and placed into spetrol photometer machine one by one.
- Within several seconds, the levels of blood total protein were shown on the digital display.
- Then record were been taken.

2.6 Method of Blood Glucose Analysis

Four generations of blood glucose meter, C. 1993 2005 sample sizes vary from 30 – 0.3ul. Test times vary from 5 seconds to 2 minutes (modern meters are typically below 15 seconds).
A blood glucose meter is an electric device for measuring the blood glucose level.

- A relatively small drop of blood was placed on a disposable test strip which interfaces with a digital meter.
- Within several seconds, the level of blood glucose was shown on the digital display.
- Then records were taken.

2.7 Method of Albumin analysis

- After separation of serum with blood plasma
- Blank tube was been prepared and also a stander tube each time analysis is to take placed.
- Six (6) tube were been prepared for each samples
- 3,000 micro liters of Albumin reagent (3mls) were been dropped in each tubes.
- 10 micro liters of blood in each sample tubes.
- 10 micro liters of albumin reagent in stander test tube.
- They were all mixed well and incubated at 30oc – 37oc for 10 minutes by water bath machine.
- The absovent were been (read) using spectrum at 578nm (nanometer)
- The stander was been pour on curvet and placed in to the machine blank.
- Then the stand was reading 0.27.
- Then each samples were be poured in curvet placed and read in g/dl

2.8 Method of Hemoglobin

- By minus total protein figure with Albumin figure
- The remaining figure is your hemoglobin.

2.9 Data Analysis

The data obtained was summarized as mean and analysis of variance (ANOVA).

3. Result and Discussion

To determining the effect of diazepam anesthesia on total blood protein and blood glucose in Goats six male adult, health West Africa dwarf goats weighing 13 to 16kg were used. Anesthesia was induced using 0.2mg/kg diazepam intravenously.

The blood glucose (mml/L) following intravenous administration of diazepam in goats.

Goat	Before Adm. of drug	2hrs after recovery	24hrs after recovery	5days after recovery	7days after recovery	Total	Mean
A	6.1mml/L	3.6mml/L	3.4mml/L	3.9mml/L	4.3mml/L	21.3	4.30
B	4.1mml/L	3.6mml/L	3.1mml/L	3.5mml/L	3.7mml/L	18.0	3.60
C	5.1mml/L	3.3mml/L	3.6mml/L	3.8mml/L	4.1mml/L	19.9	3.98
D	4.0mml/L	3.1mml/L	3.4mml/L	3.6mml/L	3.7mml/L	17.8	3.56
E	4.3mml/L	3.0mml/L	2.9mml/L	3.4mml/L	3.8mml/L	17.4	3.48
F	4.2mml/L	3.5mml/L	3.3mml/L	3.7mml/L	3.8mml/L	18.5	3.70
Total	**27.8**	**20.1**	**19.7**	**21.9**	**23.4**	**112.9**	
Mean	**4.63**	**3.35**	**3.28**	**3.65**	**3.90**		

Total protein (g/dl) of blood following intravenous administration of diazepam in goats.

Goat	Before Adm. of drug	2hrs after recovery	24hrs after recovery	5days after recovery	7days after recovery	Total	Mean
A	5.5g/dl	4.5g/dl	5.2g/dl	5.6g/dl	5.3g/dl	26.1	5.22
B	5.1g/dl	5.5g/dl	5.0g/dl	5.1g/dl	5.0g/dl	25.7	5.14
C	5.8	4.8	5.8	5.0	4.9	26.3	5.30
D	5.5	5.2	6.0	5.8	5.2	27.7	5.54
E	6.0	5.8	4.7	4.9	5.1	26.5	5.30
F	5.3	6.3	5.2	5.2	5.2	27.2	5.44
Total	**33.2**	**32.1**	**31.9**	**31.6**	**30.7**	**159.5**	
Mean	**5.53**	**5.35**	**5.32**	**5.26**	**5.11**		

Hemoglobin (g/dl) of blood following the intravenous administration of diazepam in goats.

Goat	Before Adm. of drug	2hrs after recovery	24hrs after recovery	5days after recovery	7days after recovery	Total	Mean
A	1.2g/dl	1.6g/dl	1.3g/dl	1.6 g/dl	1.2 g/dl	6.9	1.38
B	1.1g/dl	1.9g/dl	1.4 g/dl	1.2 g/dl	1.1 g/dl	6.7	1.34
C	1.5g/dl	1.0 g/dl	1.9 g/dl	1.7 g/dl	1.1 g/dl	7.2	1.44
D	1.1g/dl	1.3 g/dl	1.7 g/dl	1.7 g/dl	1.1 g/dl	6.9	1.38
E	1.4 g/dl	1.7 g/dl	1.6 g/dl	1.4 g/dl	1.4 g/dl	7.5	1.50
F	1.4 g/dl	1.9 g/dl	1.3 g/dl	1.3 g/dl	1.3 g/dl	7.2	1.44
Total	**7.7**	**9.4**	**9.4**	**8.9**	**7.2**	**42.4**	
Mean	**1.28**	**1.56**	**1.56**	**1.48**	**1.2**		

Albumin (g/dl) of blood following intravenous administration of diazepam in goats.

Goat	Before Adm. of drug	2hrs after recovery	24hrs after recovery	5days after recovery	7days after recovery	Total	Mean
A	4.3g/dl	2.9g/dl	3.9g/dl	4.0 g/dl	4.1 g/dl	19.2	3.84
B	4.0g/dl	3.6g/dl	3.6 g/dl	3.9 g/dl	3.9 g/dl	19.0	3.80
C	4.3g/dl	3.8 g/dl	3.9 g/dl	3.3 g/dl	3.8 g/dl	19.1	3.82
D	4.4g/dl	3.9 g/dl	4.3 g/dl	4.1 g/dl	4.1 g/dl	20.8	4.16
E	4.6 g/dl	4.1 g/dl	3.1 g/dl	3.5 g/dl	3.7 g/dl	19.0	3.80
F	3.9 g/dl	4.4 g/dl	3.9 g/dl	3.9 g/dl	3.9 g/dl	20.0	4.00
Total	**25.5**	**22.7**	**22.7**	**22.7**	**23.5**	**117.1**	
Mean	**4.25**	**3.78**	**3.78**	**3.78**	**3.91**		

4. Discussions

4.1 Effects of diazepam on the blood glucose

The glucose analyses were performed before (To) anesthesia, 2 hours, 24 hours, 5 days and 7 days after recovery.

In control (To) the average blood glucose was within the normal range (3.6 to 5.8mml/L) but after administration of diazepam, at 2 hours, 24 hours, 5 days and 7 days were decreased in glucose level throughout the experiments.

This means the diazepam have negative effect on blood glucose when compared with control value.

4.2 Effects of Diazepam on The Total Protein

a. Effects of diazepam on the albumin.

The albumin analyses were performed before (To) anesthesia, 2 hours, 24 hours, 5 days and 7 days after recovery.

In control (To) the average albumin value within the normal range (3.5 to 5.0g/dl). Before (To) administration albumin value was 4.3g/dl and after administration of diazepam at 2 hours, 24 hours 5 days of recovery, albumin value were decreased (3.78g/dl, 3.78g/dl and 3.78g/dl) while 7 days after recovery albumin value (3.91g/dl) had started returned to the control (To) value. Thus diazepam had negative effect on the albumin by decreased it when compared with control value.

b. Effects of diazepam on the hemoglobin

The Hemoglobin analyses were performed before (TO) anesthesia, 2 hours, 24 hours, 5 days and 7 days after recovery.

In control (To) the average Hemoglobin value was within the normal range (1. 0 to 1.5g/dl). After administration of diazepam at 2 hours, 24 hours and 5 days of recovery, hemoglobin values were increased.

The results shown that before (To) administration of the drug, hemoglobin level was 1.3g/dl while 2 hours and 24 hours of recovery, hemoglobin value were 1.6g/dl, 1.6g/dl which shown significant increased while in 5 day (1.5g/dl) and 7 days (1.2g/dl) of recovery, the hemoglobin value decreased when compared with the control (To) vaule, even though there was minor increased at 2 hours, 24 hours and minor decreased at 5 days and 7 days lower to the control value.

5. Conclusion

The result of these analysis revealed that administering of diazepam at 0.2mg/kg before and after administration there was not significant increased in hemoglobin, total protein and albumin while blood glucose indicate that there was a significant decrease in blood glucose percentage ($P \geq 0.05$) at 2 hours and 24 hours after recovery.

6. Recommendation

Further studies with this drug in goats is recommended in order to maintain normal total blood protein and blood glucose either surgery or any research work, so as to enable its clinical application.

A specific antagonist is also recommended in other to reverse the effect of the drug e.g. yohumbine, tolazobrine, aminopyridine, flumazeni etc.

Finally, the concerns for the practicing veterinarian include:

- Carefully evaluating the physical condition of the animal pre-procedure.
- Preventing regurgitation & ruminal tympany during the procedure.
- Monitoring & supportive care to assure suitable intubation, respiration, blood pressure and heart rate.
- Appropriate drug administering for induction & maintenance of general & local anesthesia, as well as postoperative analysis and withdrawal time.

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