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Research Article

A Clinico-Biochemical Study of Diazepam as a Preanesthetic in Combination with Various Anesthetics During Orchidectomy in Dogs

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Abstract | Dog has been a companion animal for humans since years. Being kept in a variety of environments dogs are prone to injuries, wounds and trauma that might require the use of anesthesia to repair and treat the animals. Injectable anesthetics are preferred over inhalant anesthetics in animals as the later require a complicated technique and mechanical instruments. So, the injectable anesthetics are preferred in dogs. Diazepam with Propofol and Diazepam with Ketamine HCl are important anesthetic combinations for induction. Ketamine HCl is a type of dissociative anesthesia has been used for years. Propofol unaided is used as general anesthesia and causes rapid induction provide sedation during anesthesia. To overcome the apnea caused by alone Propofol can be overcome by using combination of Diazepam with Propofol. At present, Diazepam with Ketamine HCl combination can also be used for better results. A total of 12 clinically healthy mongrel dogs were arranged and kept in experimental shed at Surgery Teaching Hospital University of Veterinary and Animal Sciences (UVAS), Ravi Campus, Pattoki. The dogs were placed in two group i-e. Group A and B. the animals were housed under normal conditions for duration of one week. Each dog of group A was administered Diazepam (Valium 2ml; Martin Dow Pharmaceuticals (Pak) LTD) with Propofol (Propofol 20ml; Sandoz Limited, Germany) combination (dose: Diazepam 0.2-0.5 mg/kg of b. wt. and Propofol 5 mg/kg of b. wt.) while each dog of group B was administered Diazepam (Valium 2ml; Martin Dow Pharmaceuticals (Pak) LTD) with Ketamine HCl (Ketamine Hydrochloride 10ml; West ward Pharmaceuticals, USA) combination (dose: Diazepam 0.2-0.5 mg/kg of b. wt. and Ketamine HCl 10-15 mg/kg of b. wt.). Physical parameters like temperature, pulse and respiration, Clinical Parameters like Palpebral reflexes, Pedal withdrawal reflex, degree of analgesia, degree of sedation, induction of anesthesia and ease of recovery was evaluated at different time intervals. LFTs (ALP, AST, ALT and TB) and RFTs (Creatinine and BUN) were performed to find out the effect of anesthesia on liver and kidney. Orchidectomy in dogs was performed after the anesthesia has been achieved and then the post-operative care was done for 5 days after surgery. It is concluded from this research that Diazepam with Ketamine HCl shows an ideal, safe, cost effective and easily available anesthetic with minimal hazards to perform minor and major surgical exercises on small animals.

Keywords | Diazepam, Ketamine HCl, Propofol, Orchidectomy, Anesthesia

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INTRODUCTION

Dog has been a cohort animal for humans since centuries and this relationship between humans and dogs keeps on growing stronger and stronger along with the people interest is also keep on going towards the care of animals. It has been used not only as guard but also to helping in daily routines and especially as companion animals. From

times till now dogs are of utmost importance in human life and were used as creature for hunting and animal for burden and now as a pet animal also (Jafari and Emam-Djomeh, 2007). Being kept in a verity of environment dogs are prone to injuries, wound and trauma which might require the use of anaesthetic for the repair and treatment of the conditions. Sometime highly painful procedures are required for injuries and operations. Anesthetics are used for both inhalational and parenteral routes for the surgery in dogs. Moreover, anesthetics can be classified into intramuscular and intravenous depending upon their route and ease of administration. People used ice for analgesia in ancient times and with the passage of time Ketamine Hydrogen Chloride (HCl) was introduced as a first injectable anesthetic in 1962 and accepted for usage in the United States in 1970. Ketamine HCl performs as specific antagonist of the receptor N-Methyl-D-aspartic acid NMDA, an inotropic glutamate base receptor (Anis et al., 1983). It induces analgesia, sedation, and amnesia (memory loss). Effects characteristically begin in five minutes when administered by injection with the main effects durable up to 25 minutes (Mair et al., 2009). Propofol is sole ultra-short acting intravenous, non-steroid and non-barbiturate anesthetic agent (Hofmeister et al., 2006). It is linked with a fast-smooth induction and a quick retrieval after the surgical intervention has been performed (VanNatta and Rex, 2006). It might be specified in assured clinical cases with cardiovascular depression (Haskins et al., 1986; Kolata, 1986; Hellyer et al., 1991; White et al., 2001; Boutureira et al., 2007; Hazra et al., 2008; Beteg et al., 2010). Diazepam and the Ketamine HCl induction of anesthesia and recovery features related with the Propofol have not been stated before (Seliskar et al., 2007). The goal of this potential clinical study is to relate Diazepam with Propofol combination verses Diazepam with Ketamine HCl for the induction of anesthesia and recovery. The mixture of Diazepam with Propofol at a dose rate of 0.2 – 0.5 mg/kg and 10 – 15 mg/kg correspondingly, is normally related with eagerness free induction of anesthesia in dogs.

Orchidectomy is usually performed at the age of 6 months, to avoid breeding in unwanted dogs. The foundation of this viewpoint is typically in the attention to control pets' population (Hart et al., 2016). Besides this, this intervention is also advised to prevent male dog from testicular cancer, reduction of prostate problems including prostate cancer and modification of behaviors of dog. This procedure as a major surgery requires use of ideal anesthesia to reduce the intra-operative pain. To check the effects of anesthesia on the body of animal by biochemical analysis like renal function test and liver function tests was carried out. The liver function tests (Serum alkaline phosphatase ALP, serum alanine transaminase ALT, and serum aspartate transaminase AST) and renal function tests (Total bilirubin level, BUN and Albumin concentrations) was performed (Rand et al., 1991) before and after the administration of anesthesia to figure out the effects of anesthesia on liver and kidney.

MATERIALS AND METHODS

The current study was performed on twelve dogs ranging between 1-3 years of age and weighing 25-40 Kg and kept under normal conditions for about a week before start of research at Surgery Teaching Hospital, University of Veterinary and Animal Sciences, Ravi Campus Pattoki. Before surgical interventions, dogs were evaluated physically for age, weight and physical health as well as responses. Finally, pre-anesthetic evaluation was carried out to evaluate the animal fit for anesthetic administration and further surgical interventions.

Experimental design

A total of twelve (12) dogs were selected and divided into following two groups; Group A: 1A, 2A, 3A, 4A, 5A, 6A while Group B: 1B, 2B, 3B, 4B, 5B, 6B

The present project was designed to evaluate the two combinations of anesthesia as an inducing agent which influences the superiority of induction, recovery in clinically healthy dogs experiencing anesthesia during orchidectomy and effect on liver and kidneys performing LFTs and RFTs as well before and after the administration of drugs.

Preoperative considerations

All dogs were completely examined physically and clinically to rule out any pre-operative problem and all the surgical protocols were adopted prior to surgery.

Experimental design

Six dogs were allocated for this group. Animals were restrained and were administered with pre-anesthetic diazepam at the dose rate of 0.1-0.2 mg/kg intramuscular (Slatter, 2003) and catheterized intravenously to administer combination of Diazepam with Ketamine HCl for the induction of anesthesia. Each dog of group A was administered with Diazepam and Ketamine HCl combination dose: Diazepam (Valium 2ml; Martin Dow Pharmaceuticals (Pak) LTD) at 0.2-0.5 mg/kg of b. wt. (Van der Kleijn et al., 1971; Frey et al., 1984; Ferreira et al., 2015) and Ketamine HCl (Ketamine Hydrochloride 10ml; West ward Pharmaceuticals, USA) 10-15 mg/kg of b. wt. (Van der Kleijn et al., 1971; Frey et al., 1984; Ferreira et al., 2015). After sedation animal was placed on opreation table for surgery. Six dogs were allocated for this group. Animals were restrained and were administered with pre-anesthetic diazepam at the dose rate of 0.1-0.2 mg/kg intramuscular (Slatter, 2003) and catheterized intravenously to administer combination of Diazepam with Propofol for the induction

of anesthesia. Each dog of group B was administered Diazepam and Propofol combination (dose: Diazepam (Valium 2ml; Martin Dow Pharmaceuticals (Pak) LTD) 0.2 – 0.5 mg/kg of body weight (Van der Kleijn et al., 1971; Frey et al., 1984; Ferreira et al., 2015) and Propofol (Propofol 20ml; Sandoz Limited, Germany) 5 mg/kg of body weight (Ferreira et al., 2015; Slatter, 2003). After sedation animal was placed on table for surgery.

Parameters evaluated

Observations were recorded for different parameters like;

Physical parameters

Temperature has been taken from anus of every animal of both groups in degree Celsius at different time intervals of 0, 15, 30, 45 and 60 minutes (Hansen, 2003). Pulse has been taken from femoral artery of every animal of both groups in beats per minute at different time intervals of 0, 15, 30, 45 and 60 minutes (Hansen, 2003). Respiration rate has been taken from lungs by using stethoscope of every animal of both groups in beats per minutes at different time intervals of 0, 15, 30, 45 and 60 minutes (Hansen, 2003).

Clinical parameters

Dogs were observed for body responses according to the score card defined by (Ibrahim, 2017). Pedal withdrawal reflex of dogs was tested by score card assigned by (Ibrahim, 2017). This parameter was checked by assigning scores to dogs' reflexes of Toe pinching and Tail Response as defined by (Breivik et al., 2008). Four scores were assigned to test the degree of sedation in dogs using the references like palpebral response set by (Mondello et al., 2002). Four scores were assigned to this parameter depending upon various responses and reflexes of animal as defined by (White and Yates, 2017). Ease of recovery from anesthetic was checked using the parameters/scores defined by (White and Yates, 2017) for reappearance of consciousness or reflexes.

Blood collection

The blood samples were collected at two different intervals; before and after surgery, directly from cephalic vein that was located in the front leg of dog. Blood samples were kept in gel containing vacutainer (yellow Lid) for hematological parameters and biochemical evaluation.

Liver function test

- Alkaline Phosphatase (ALP)
- Alanine Aminotransferase (ALT)
- Aspartate Amino Transferase (AST)
- Total Bilirubin (TB)

Renal function test

- Creatinine
- Blood Urea Nitrogen (BUN)

Surgical procedure of neutering

The anesthetized dogs were applied with Povidone iodine surgical scrub at surgical site for complete sterilization. Scrubbing was done in the circular manner from the center towards periphery. Two drape layers were used; first layer contained four drapes and they were placed over the animal. The second layer contained one big drape with central slit place over first layer.

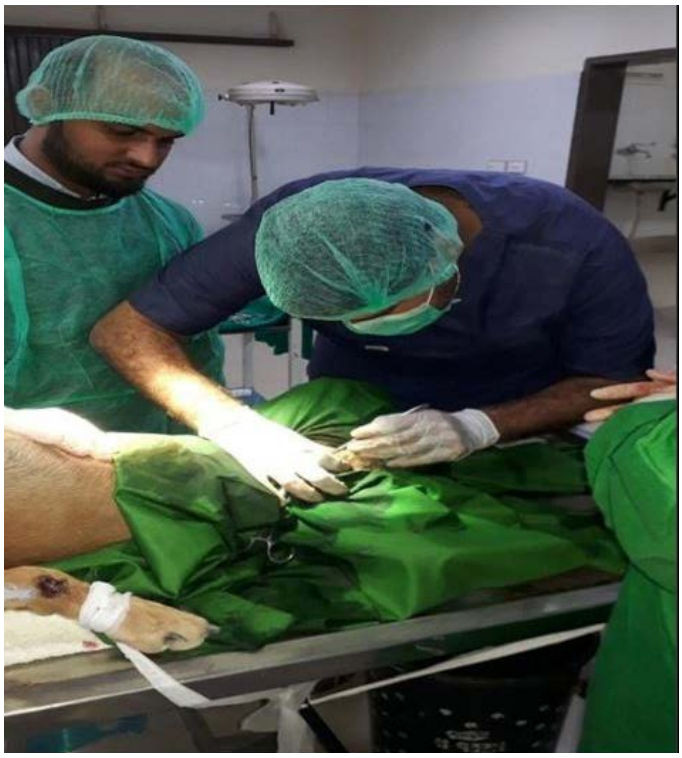

Figure 1: Incision on scrotum

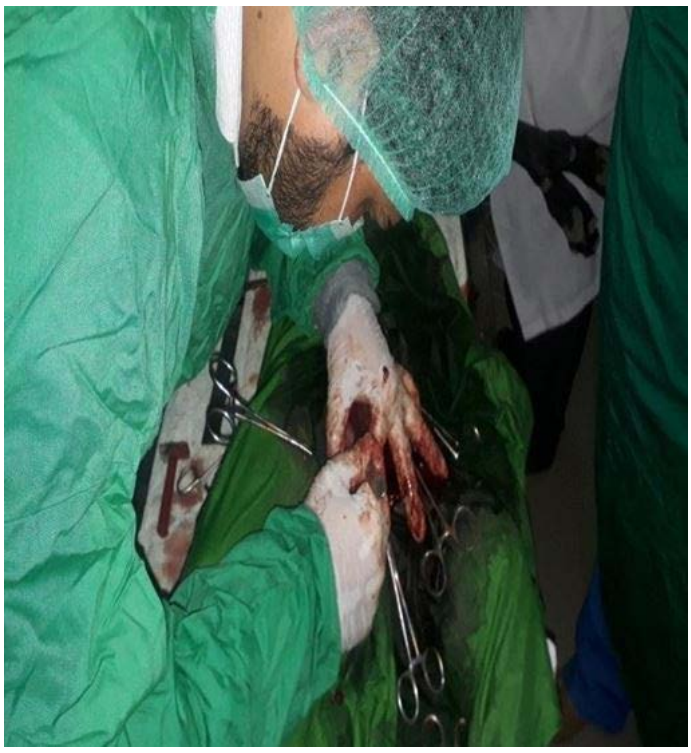

Figure 2: Retraction of testes

Pre-scrotal approach was used for performing neutering in all dogs. A skin and subcuticular tissue incision was made in front of the scrotum (pre-scrotal) over the shaft of the penis to expose the testicles. The scrotum was on the left under the hand. We used, "open procedure" and a tie or ligation was placed around internal cremaster, pampiniform plexus and vas deferens. When both testicles were removed then the skin incision was closed by using absorbable suture material (Vicryl#1) to close the sub-cutaneous tissue by using simple continuous suture pattern and skin was closed with simple interrupted pattern using non-absorbable (Glysilk#1) suture material. The same surgical procedure was done in other dogs of both groups by using above designed anesthetic combination and the effects of these pre-anesthetic mixtures were checked on hematological liver and kidney functions.

Figure 3: Simple continuous and simple interrupted suture pattern on sub-cutaneous.

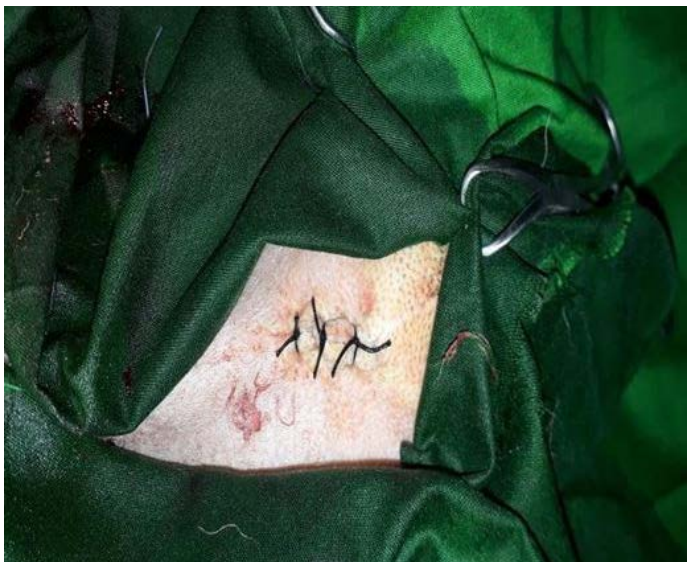

Figure4: Skin tissues using absorbable and non-absorbable suture material respectively.

Post-operative considerations

After surgical procedure, the dogs were kept in cages of Surgery Teaching Hospital under complete observation and care. Postoperative care was provided to each dog accordingly in the cages. Analgesics were given for five days inj. Prefen (Ketoprofen–Fynk Pharmaceuticals) at the dose rate of 0.2-1 mg/Kg body weight up to 5th day of operation. Antibiotic coverage was given with inj. Velosef (Cephradine - GlaxoSmithKline) at the dose rate of 25mg/Kg body weight up to 5th day of operation.

RESULTS

Physical parameters

The effects of anesthetic combinations were evaluated focusing variations in physiological parameters and the results of clinical parameters including temperature, pulse and respiration are presented in Table 1.

Table 1 shows statistical analysis of Physical parameters (Temperature, Pulse, Respiration) of the animals between Group A and Group B at different intervals of 0 mint, 15 mint, 30 mint, 45 mint and 60 mint respectively through Paired sample T test which showed a statistical non-significance between groups (P>0.05) for the parameter of Temperature at all the time intervals. There was a statistical significance (P<0.05) at all the time intervals except at 0 mint for Pulse while the results of respiration were statistically significant (P<0.05) at all the time intervals for both groups.

Clinical parameters

Reflexes of animals were also evaluated by two parameters; Palpebral Reflex and Pedal Reflex and the results are tabulated below.

Group A and group B showed a statistical non significance P=0.07 for palpebral reflex when analyzed through paired T test i.e. (P > 0.05) as shown in Table 2.

- Score 1: No alteration in reflex
- Score 2: Moderate reflex
- Score 3: Lethargic reflex
- Score 4: Absences response

Group A and group B showed a statistical non significance P=0.07 for pedal reflex when analyzed through paired T test i.e.(P>0.05) as shown in Table 3

- Score 1: No limb extraction at the lock of 3rd ratchet of hemostat braced on web
- Score 2: Limb extraction at the lock of 3rd ratchet of hemostat braced on web
- Score 3: Limb extraction at the lock of 2nd ratchet of hemostat braced on web
- Score 4: Limb extraction at the lock of 1st ratchet of hemostat braced on web

Table 1: Statistical analysis of physical parameters at different time intervals.

Physical parameters	Mean±S.E.	0 mint	15 mint	30 mint	45 mint	60 mint
Temperature	Group A	102.4 ± 1.08	102.1 ± 1.06	101.6 ± 1.26	101.5 ± 1.07	101.3 ± 1.04
	Group B	102.0 ± 0.99	101.8 ± 0.98	101.6 ± 0.95	101.4 ± 1.04	101.4 ± 1.04
	P Value	0.65	0.69	1.00	0.94	0.95
Pulse	Group A	100.66 ± 7.76	96.33 ± 8.11	93 ± 7.89	90.16 ± 7.78	89.16 ± 7.78
	Group B	87.16 ± 8.60	81.33 ± 8.57	76.33 ± 8.91	72.50 ± 8.80	70.16 ± 8.42
	P Value	0.06	0.04*	0.03*	0.02*	0.01*
Respiration	Group A	25.33 ± 1.63	24.00 ± 1.54	23.00 ± 1.54	22.00 ± 1.54	21.83 ± 1.47
	Group B	22.16 ± 1.47	20.16 ± 1.47	18.16 ± 1.47	16.16 ± 1.47	15.16 ± 1.47
	P Value	0.02*	0.01*	0.00**	0.00*	0.00**

Table 2: Statistical analysis of palpebral reflex between groups.

Palpebral Reflex				
Group A	Dog No	Score	Mean ± S.E	P Value
	A1	3	2.83 ± 0.40	0.07
	A2	3		
	A3	3		
	A4	2		
	A5	3		
	A6	3		
Group B	B1	3	3.33 ± 0.51	
	B2	3		
	B3	4		
	B4	3		
	B5	4		
	B6	3		

Table 3: Statistical analysis of Pedal Reflex between groups.

Pedal reflex				
Group A	Dog No.	Score	Mean ± S.E	P Value
	A1	3	2.83 ± 0.40	0.07
	A2	3		
	A3	3		
	A4	2		
	A5	3		
	A6	3		
Group B	B1	3	3.33 ± 0.51	
	B2	3		
	B3	4		
	B4	3		
	B5	4		
	B6	3		

Table 4 shows the statistical analysis between Group A and Group B at different time interval of 0 mint, 15 mint, 30 mint, 45 mint and 60 mint which indicate the statistical significance between groups analyzed through paired sample T test, there is no statistical significance between groups at different time interval (P>0.05), but the Mean ± S.E. of two groups showed statistical significance P=0.04 (P<0.05) between groups for overall degree of analgesia when compared through repeated measures ANOVA.

Table 5 shows the statistical analysis between Group A and Group B at different time interval of 0 mint, 15 mint, 30 mint, 45 mint and 60 mint which indicate the statistical significance between groups only at 15 mint i.e. P=0.02 (P<0.05) when analyzed through paired sample T test and the Mean ± S.E. of two groups showed statistical significance P=0.02 between groups for overall ease of recovery compared through repeated measures ANOVA.

The statistical analysis of induction of anesthesia between group A and group B by using paired T test showed a high statistical significance between groups i.e. (P<0.05) as shown in Table 6.

- Score 1: Very even, with slow easing
- Score 2: Good, some coughing and swallowing
- Score 3: Deprived coughing and swallowing
- Score 4: Very deprived coughing and swallowing

Ease of recovery

Group A and group B showed high statistical significance P=0.01 for degree of sedation when analyzed through paired T test i.e.(P>0.05) as shown in Table 7.

- Score 1: Very smooth, no eagerness
- Score 2: Even, some eagerness
- Score 3: Deprived, persistent eagerness on recovery
- Score 4: Very poor

Bio-chemical parameters

Liver function test (LFT)

Different parameters of LFT (ALP, ALT, AST, TB) were statistically analyzed by Paired T test pre and post operatively as shown in Table 8. There is a statistical non significance between the groups for ALP, AST and total Bilirubin pre-operatively (P>0.05), but they

are statistically significant post operatively (P<0.05). When two groups were compared for ALT, they showed statistical significance between groups (P<0.05) both pre and post operatively.

Table 4: Statistical analysis of Degree of Analgesia between groups.

Mean±S.E	0 mint	15 mint	30 mint	45 mint	60 mint	P Value
Group A	2.00 ± 0.00	1.16 ± 0.40	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	0.04*
Group B	2.16 ± 0.40	1.83 ± 0.40	1.16 ± 0.40	1.00 ± 0.00	1.00 ± 0.00	
P Value	0.36	0.10	0.36	-	-	

Table 5: Statistical analysis of Degree of Sedation of anesthesia between groups.

Mean ± S.E	0 mint	15 mint	30 mint	45 mint	60 mint	P Value
Group A	2.00 ± 0.00	1.16 ± 0.40	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	0.02*
Group B	2.16 ± 0.40	1.83 ± 0.40	1.13 ± 0.51	1.00 ± 0.00	1.00 ± 0.00	
P Value	0.36	0.02*	0.17	-	-	

Table 6: Statistical analysis of Induction of Anesthesia between groups.

Induction of Anesthesia				
Group A	Dog No	Score	Mean ± S.E.	P Value
	A1	3	2.83 ± 0.40	0.00**
	A2	2		
	A3	3		
	A4	3		
	A5	3		
	A6	3		
Group B	B1	1	1.16 ± 0.40	
	B2	1		
	B3	2		
	B4	1		
	B5	1		
	B6	1		

Renal function test (RFT)

Table 9 shows the pre and post-operative comparison of different parameters of RFT (Creatinine and Blood Urea Nitrogen) analyzed through Paired T test which indicated a statistical non-significance between the groups for all the parameters for Creatinine (P>0.05) while shows significant results between groups for BUN (P<0.05).

DISCUSSION

Anesthesia is a crucial requirement for the surgical interferences in animals as well as in human and that's why the surgeon can do surgical interference with accuracy and wisdom. Without the miracle of anesthesia, the surgical manipulations would have been impossible. The synergistic outcome of two or more drugs can lessen the dose of both primary to price efficiency of the practice which is of inordinate apprehension in animals (Pypendop and Ilkiw, 2005). Inhalant anesthetics have cardiovascular depressant effect in small animals so balanced anesthesia is used mostly to reduce the requirement of inhalant anesthetics. Therefore, a concept of balanced anesthesia is important that includes a combination of various sedative, anesthetic agents and analgesics. Diazepam with Ketamine HCl and Diazepam with Propofol are important anesthetics for induction. Propofol alone is used as general anesthesia and causes rapid induction provide sedation during anesthesia. To overcome the apnea caused by alone Propofol can be overcome by using combination of Diazepam with Propofol. At present, Diazepam with Ketamine HCl combination can also be used. Orchidectomy in dogs was performed in dogs after the anesthesia has been achieved.

Table 7: Statistical analysis of Ease of Recovery between groups.

Ease of Recovery				
Group A	Dog No	Score	Mean ± S.E	P Value
	A1	1	1.16 ± 0.40	0.01*
	A2	1		
	A3	2		
	A4	1		
	A5	1		
	A6	1		
Group B	B1	2	2.16 ± 0.40	
	B2	3		
	B3	2		
	B4	2		
	B5	2		
	B6	2		

The present project was designed to relate the two blends of anesthesia as an inducing agent which influences the superiority of induction, recovery in clinically healthy dogs experiencing anesthesia during orchidectomy and effect on liver and kidney performing LFTs and RFTs before and

after the administration of drugs. For this purpose, twelve dogs ranging between 1-3 years of age and weighing 25-40 Kg and were kept under normal conditions for about a week before start of research at University of Veterinary and Animal Sciences, Ravi Campus Pattoki. A total of twelve (12) dogs were selected and were divided into following two groups; Group A and Group B having 6 animals each. Efficacy of Diazepam with Ketamine and Propofol was assessed on the basis of physiological, clinical and biochemical parameters in dog. Slight reduction in rectal temperature of treated dogs was observed with all of the selected doses of diazepam Ketamine group and same observation was observed in other treatment group of diazepam Propofol. On statistical analysis of both groups by using Paired T test the results were non-significant at 0 mints, 15 mints, 30 mints, 45 mints and 60 mints having P value 0.65, 0.69, 1.00, 0.94 and 0.95 respectively (P>0.05) providing the information that there is no effect on temperature of the treatments similar observation was observed by Hansen and Bernie (2003). Diazepam induces hypothermia and allows better maintenance of body temperature due to peripheral vasoconstriction by central distribution of blood. By administering intravenously the anesthesia there was a drop in pulse rate associated with peripheral vasoconstriction. This vasoconstriction was followed by vasodilation which is in line with the pattern observed by (Hansen, 2003). On analysis by using paired T test for both groups the result was significant (P<0.05) at 15 mints, 30 mints, 45 mints and 60 mints but non-significant having P value 0.04, 0.03, 0.02 and 0.01 respectively while 0.06 (P>0.05) at 0 mint. By using Propofol as an anesthetic agent either in combination or alone results into induction apnea which shows that there will be decrease in respiration rate on induction and same observation was observed during respiration analysis in group B i.e. Diazepam and Propofol. On statistical analysis of both groups results were significant at 0 mints, 15 mints, 30 mints, 45 mints and 60 mints having P value 0.02, 0.01, 0.00, 0.00 and 0.00 respectively (P>0.05) statistically ensuing that the Diazepam Ketamine provides better results than Diazepam Propofol.

Anatomic extent of anesthesia was evaluated by checking palpebral and pedal reflex after 20 minutes of the administration of anesthesia. At 20 minutes of post drug administration, maximum dogs of Group B show absence of response while other shows lethargic response on checking of palpebral reflex while there was lethargic to moderate response in dogs of group A. statistical Analysis by using paired T test for Palpebral reflex between both groups reveals that the result was non- significant P value 0.07 (P>0.05) meaning that there is no difference on evaluating palpebral reflex between both groups. Pedal reflex was checked after 20 minutes in both groups which reveals that group Diazepam with Ketamine shows reflex of limb extraction at the lock of 2nd ratchet of hemostat braced on web and a few shows reflex of limb extraction at the lock of 1st ratchet of hemostat braced on web while diazepam Propofol shows limb extraction at the lock of 3rd ratchet of hemostat braced on web. When both groups were statistically analyzed by using Paired T test the results shows that there was non- significance P value 0.07 (P>0.05) result between the groups hence showing that no difference in pedal reflex of both the animals as depicted by (Hansen, 2003).

Table 8: Statistical analysis for different bio-chemical parameters of LFT.

Parameters (LFT)	Mean ± S.E	Pre-operative	Post-operative
ALP	Group A	55.88 ± 10.73	96.61 ± 4.27
	Group B	66.41 ± 8.58	106.76 ± 7.44
	P Value	0.09	0.04*
ALT	Group A	26.77 ± 2.28	31.95 ± 3.14
	Group B	21.34 ± 1.07	58.37 ± 2.27
	P Value	0.00**	0.00**
AST	Group A	25.65 ± 4.00	59.38 ± 3,35
	Group B	25.85 ± 5.49	42.93 ± 3.88
	P Value	0.95	0.00**
Total Bilirubin	Group A	0.38 ± 0.03	0.51 ± 0.02
	Group B	0.36 ± 0.04	0.56 ± 0.08
	P Value	0.34	0.23

Table 9: Statistical analysis for different bio-chemical parameters of RFT.

Parameters (RFT)	Mean±S.E	Pre-operative	Post-operative
Creatinine	Group A	0.49 ± 0.08	0.65 ± 0.09
	Group B	0.58 ± 0.21	0.70 ± 0.15
	P Value	0.17	0.45
BUN	Group A	9.26 ± 3.00	16.38 ± 2.53
	Group B	12.19 ± 2.53	20.41 ± 2.41
	P Value	0.04*	0.00**

Degree of analgesia and degree of sedation was analyzed between group A and B by using scoring system as per analysis of Ibrahim (2017) which shows that propofol combination with diazepam shows mild to No degree of analgesia as compared to other group of Diazepam Ketamine as propofol have no or least analgesic property as told by of Ibrahim (2017). On statistical analysis of both groups by using paired T test the result was highly significant P=0.00 (P<0.05) statistically. Degree of sedation was examined by using score system and results showed that in the group Diazepam Ketamine the animal were co-operative during surgery and handing while were

restless or nervous during surgery or handling when the combination of Diazepam Propofol was administered hence showing that the result was significant P= 0.01 (P<0.05) which provides the information that the effect of Diazepam Ketamine combination have a better effect on degree of sedation than Diazepam and Propofol combination. Induction of anesthesia was a clinical parameter that was checked by scoring system as analyzed by Ibrahim (2017) and revealed the result at 0 mints, 15 mints, 30 mints, 45 mints and 60 mints having P value 0.36, 0.10, 0.36, 1.00 and 1.00 respectively showing that the result was non-significant (P>0.05) when both groups i.e. Diazepam Ketamine and Diazepam Propofol were examined at different time intervals but the induction in the group of Diazepam Ketamine was quite smooth and animal were calm while in the Diazepam Propofol group the animal were smooth in induction but one of them shows ranching. Ease of recovery was another parameter that was analyzed by scoring system as the others were examined. On statistical evaluation the results showed that the results at 15 minutes after the induction was significant P= 0.02 (P>0.05) while non-significant at 0 mints, 30 mints, 45 mints and 60 mints having P values 0.36, 0.17, 1.00 and 1.00 (P>0.05). the recovery was very smooth in the group of Diazepam and ketamine with normal behavior of the animal while animal recovery was seen in Diazepam Propofol group with a mild degree sedation.

Liver and kidney play a key role in the body especially when we have to discuss bout anesthesia and anesthesia have effect on both organs. To evaluate the effect of different anesthetics the evaluation of kidney and liver can be done by evaluating different tests like Liver function test (LFTs) and Renal function test (RFTs). First of all, the effect of anesthetics can be evaluated by LFTs by having different test like (Serum alkaline phosphatase ALP normal range is about 10.6 to 101 u/L, alanine transaminase ALT normal range is about 8.2 to 57 u/L, aspartate transaminase AST normal range is about 8.9 to 49 u/L and Total Bilirubin normal range is about 0.2-0.6 mg/dl) were carried out before and after administration of anesthesia to find out the effect of anesthesia on liver. On evaluation before and after surgery has been performed the results revealed that the effect of Diazepam and Ketamine have least effect on liver as compared to the Group having combination of Diazepam and Propofol. The P value for the ALP, ALT, AST and TB were 0.09, 0.00, 0.95 and 0.34 respectively showing that ALP, AST and TB have no difference in both groups before administration of anesthesia and were same while ALT have a little bit difference before drugs administration showing that (P>0.05). Results showed that after the administration of Diazepam and Ketamine combination and Diazepam Propofol combination, the P values for the ALP, ALT, AST and TB were 0.04, 0.00, 0.00, 0.23 respectively revealing that the effect of Diazepam and ketamine was least than Diazepam and Propofol (P<0.05). by overall results it was concluded that the effect of Diazepam and Ketamine was least than Diazepam and propofol and some results was similar discussed by Ferreira et al., 2015.

Renal Function test is used to determine the effect of anesthetics over the kidneys by taking test like Creatinine and BUN before and after surgery. The Serum was collected for RFTs which described the values for Creatinine normal range is 0.3-0.9 mg/dl and Blood Urea Nitrogen Bun normal range is about 6-24mg/dl. On analysis before and after surgery has been performed, the statistical analysis by Paired T test describes P value pre-operatively were 0.17 and 0.04 respectively showing non-significant results for Creatinine while significant result in BUN and have same values and same effects before surgery while post-operatively P values for Creatinine and BUN were 0.45 and 0.00 respectively described that the effect of Creatinine and Bilirubin were same post-operatively and have non-significant (P>0.05) results for Creatinine and Significance (P<0.05) for BUN and same results was similar discussed by Ferreira *et al.*, 2015.

CONCLUSION

Present research revealed that the anesthetic combination of Diazepam with Ketamine showed significant results for Physical Parameter (Pulse and Respiration), Clinical Parameters (Degree of analgesia, Degree of sedation, Induction of Anesthesia and Ease of recovery), Biochemical Parameters of Liver Function (ALP, ALT and AST) and Biochemical Parameters of Renal Function (BUN) when compared with Diazepam with Propofol Combination but showed non-significant results for Physical Parameter (Temperature), Clinical Parameters (Palpebral and Pedal Reflexes), Biochemical Parameters of Liver Function (Total Bilirubin) and Biochemical Parameters of Renal Function (Creatinine). From the above statistical facts it can be concluded that anesthetic combination of Diazepam and Ketamine provides safest injectable anesthetic cocktail for induction of anesthesia in dogs and is cost effective with least undesired effects on liver and Kidneys when compared with other Diazepam and Propofol.

RECOMMENDATION

It is suggested from this research that Diazepam with Ketamine HCl shows an ideal, safe, cost effective and easily available anesthetic with minimal hazards to perform minor and major surgical exercises on small animals.

AUTHORS CONTRIBUTION

Nasir Iqbal, Muhammad Arif Khan, Hammad Bin Rashid, Naveed Hussain, Mate ur Rehman Khan and Sadaf Aslam : Experimental Trial and Revision. Ayesha Sadiq, Zubair Luqman, Hamza Jawad: Formatting, Setting and Revision

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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