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A BIOCHEMICAL STUDY OF DIAZEPAM AS A PRE-ANESTHETIC IN COMBINATION WITH VARIOUS ANESTHETICS DURING ORCHIDECTOMY IN DOGS

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ABSTRACT

Diazepam as a Pre-anesthetic with Propofol or Ketamine HCl has vital combinations for the induction of anesthesia in dogs. The main objective of the study was to provide cost effective and safest injectable anesthetic cocktail for induction of anesthesia in dogs and to evaluate undesired effects on liver and kidneys of the anesthetic agent. Experimental dogs (n=12) were placed equally in two groups (A and B). Each animal from group A was administer Diazepam with Propofol blend while that of group B was given Diazepam with Ketamine HCl. Orchidectomy was performed as per standard protocols for considering as a pain module. Physical parameters like temperature ($P>0.05$), pulse ($P<0.05$) and respiration ($P<0.05$) and Biochemical parameters like renal ($P>0.05$) and liver functioning test ($P<0.05$) were performed. This research reveals that Diazepam with Ketamine HCl depicts better results over Diazepam with Propofol. It can be concluded that Diazepam with Ketamine HCl shows a safe, cost effective, ideal and easily obtainable anesthesia with least drastic hazards over kidney and liver. So, Diazepam in combination with Ketamine HCl can be used for induction of anesthesia and to execute major as well as minor surgical exercises on dogs.

***Keywords*:** Diazepam, Ketamine HCl, Propofol, Orchidectomy, Anesthesia.

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1. 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 help 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). 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 min (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 (White et al. 2001; Boutureira et al. 2007; Hazra et al. 2008). 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) were performed (Hart et al. 2016). before and after the administration of anesthesia to figure out the effects of anesthesia on liver and kidney.

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2. MATERIALS AND METHODS

This research has done by the Permission of Research Ethical Committee of University (UVAS). The current study was performed on twelve dogs ranging between 1-3 years of age and weighing 25-40kg 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.

2.1. 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. Animals were restrained and administered with pre-anesthetic diazepam at the dose rate of 0.1-0.2mg/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 0.2-0.5mg/kg bwt (van der Kleijn et al. 1971; Frey et al. 1984) and Ketamine HCl (Ketamine Hydrochloride 10ml; West ward Pharmaceuticals, USA) 10-15 mg/kg bwt (Ferreira et al. 2015).

After sedation animal was placed on 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 0.1-0.2mg/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 bwt (Ferreira et al. 2015) and Propofol (Propofol 20ml; Sandoz Limited, Germany) 5mg/kg bwt (Slatter 2003; Ferreira et al. 2015). After sedation animal was placed on table for surgery.

2.2. 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 min (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 min (Hansen 2003).

2.3. Biochemical Parameters

All the biochemical tests were performed in University Diagnostic Laboratory, University of Veterinary and Animal Sciences Lahore, Pakistan. Liver Function Tests including Alkaline Phosphatase (ALP), Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Total Bilirubin (TB) were tested by using serum sample of all the animals in both groups A and B pre-operatively and post-operatively. Renal Function Tests including creatinine and Blood Urea Nitrogen (BUN) were tested within 24 hours by using serum sample of all the animals in both groups A and B pre-operatively and post-operatively.

2.4. 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. 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.

2.5. 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 0.2-1mg/kg bwt up to 5th day of operation.

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Antibiotic coverage was given with inj. Velosef (Cephradine - GlaxoSmithKline) at the dose rate of 25mg/Kg body weight up to 5th day of operation.

3. RESULTS

3.1. 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: Statistical analysis of physical parameters (mean±SE) at different time intervals (minutes)

Physical Parameters	Groups	Time Intervals (minutes)				
		0	15	30	45	60
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 shows statistical analysis of Physical parameters (Temperature, Pulse, Respiration) of the animals between Group A (06 animals) and Group B (06 animals) at different intervals of 0-, 15-, 30-, 45- and 60-min 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 min for Pulse while the results of respiration were statistically significant ($P<0.05$) at all the time intervals for both groups.

3.2. Bio-chemical Parameters

3.2.1. 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 2. 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 2: Statistical analysis for different bio-chemical parameters of liver function tests (mean±SE)

Parameters (LFT)	Groups	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

Renal Function Tests (RFT): Table 3 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 ($P<0.05$).

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Table 3: Statistical analysis for different bio-chemical parameters of renal function tests (mean±SE)

Parameters (RFT)	Groups	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**

4. 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. 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). Diazepam with Ketamine HCl and Diazepam with Propofol are important anesthetics for induction. Orchidectomy in dogs was performed in dogs after the anesthesia has been achieved. The present project was designed to relate the two blends of anesthesia as an inducing agent which influences the physical parameters 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. 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-, 15-, 30-, 45- and 60-min 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 (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-, 30-, 45- and 60-min having P value by using paired sample T test is 0.04, 0.03, 0.02 and 0.01 respectively while non-significant at 0 minute having 0.06 ($P>0.05$). 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 ($P<0.05$) at 0-, 15-, 30-, 45-, and 60-min having P value by using paired sample T test is 0.02, 0.01, 0.00, 0.00 and 0.00, respectively statistically ensuing that the Diazepam Ketamine provides better results than Diazepam Propofol. 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. 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. Liver functioning tests including ALP, ALT, AST and TB have the pre-operative P value ($P>0.05$) by using paired sample T test 0.09 for ALP, 0.00 for ALT, 0.95 for AST and 0.34 for TB 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 ($P>0.05$) before drugs administration showing that. Results showed that after the administration of Diazepam and Ketamine combination and Diazepam Propofol combination, the post-operative P value ($P>0.05$) by using paired sample T test 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). On analysis before and after surgery has been performed, the statistical analysis by Paired T test describes P value ($P>0.05$) pre-operatively were non-significant 0.17 and significant 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 ($P>0.05$) for creatinine and BUN were non-significant 0.45 and significant 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) and biochemical parameters of liver function (ALP, ALT and AST) and biochemical parameters of renal function (BUN) when compared with Diazepam with Propofol

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Combination but showed non-significant results for physical parameter (temperature) and 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.

Compliance and ethics: The author(s) declare that they have no conflict of interest.

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