

Evaluation of General Anesthesia Using Xylazine-Ketamine Combination with and without Diazepam for Ovariohysterectomy in Bitches

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Abstract

Clinical anesthetic trial was conducted on twelve apparently healthy bitches presented to Gondar University Veterinary Clinic for ovariohysterectomy procedures. The aim of the study is to evaluate the effects of intravenous diazepam on physiological, hematological, and anesthetic parameters and also to assess anesthetic complications and develop anesthetic protocol for dogs. The bitches were randomly assigned into Group I and Group II. Anesthetic protocol was achieved by administration of atropine (0.04 mg/kg BW, S.C) immediately followed with xylazine-ketamine (1 mg/kg BW+10 mg/kg BW, I.M) for both groups but diazepam (0.5 mg/kg BW, I.V) was also given to Group I bitches when the bitches were attaining lateral recumbency. Physio-hematological and anesthetic parameters were recorded and analyzed. Quality of induction and recovery was statistically significant ($P<0.05$) difference between the groups. The mean of induction time was significantly ($P<0.05$) shorter in Group I. The mean time for loss of pedal reflex was found significantly ($P<0.05$) decreased in group I. Response to surgical incision and muscle relaxation was statistically significant ($P<0.05$) difference between groups. Duration of anesthesia, time of sternal recumbency, time of unassisted standing and duration of recovery were significantly ($P<0.05$) longer in Group I as compared Group II. Post anesthetic salivation was significantly ($P<0.05$) exhibited by Group I. No bitches were died during anesthesia or after recovery. In conclusion, atropine-Xylazine-ketamine-diazepam anesthesia does not affect physio-hematological parameter and is a very satisfactory anesthetic protocol for excellent induction, adequate muscle relaxation, longer duration of anesthesia and smooth recovery compared to atropine-Xylazine-ketamine anesthesia.

Keywords: Atropine; Bitch; Diazepam; General anesthesia; Ketamine; Ovariohysterectomy; Xylazine

Introduction

Anesthesia is a reversible process which is targeted to produce a convenient, safe, effective, yet inexpensive means of chemical restraint so that medical or surgical procedure may be conducted with minimum stress, pain, discomfort, and toxic side effects to the patients or to the anesthetist [1,2].

The selection of anesthetic drugs and techniques depends on species, breed, age, physical status, concurrent medications of the animal, personal knowledge and experience, availability and training of assistants, familiarity with available equipment, and length and type of operation or procedure performed. Pre-anesthetic medications are an essential part of safe anesthetic management and they minimize stress, cardiopulmonary depression, and the deleterious effect when used appropriately and associated with many intravenous (IV) and inhalation anesthetics [3].

Various sedatives and tranquilizing agents are used as pain killers and/or muscle relaxants while animals undergo minor or major surgeries. These drugs are needed in veterinary practice and are indispensable as they help in overcoming resistance of the animals during examination, maintaining depth of anesthesia, reducing the amount of anesthetic agents and increasing margins of safety. For these purposes, the commonest drugs used are Ketamine, Diazepam, Xylazine and Atropine sulphate [4].

Atropine is most commonly used in premedication in combination with acepromazine maleate to minimize or prevent vagal effects that may induce bradycardia and also this drug reduce potential muscles spasm gastrointestinal motility and secretion, salivation and animal respiratory secretion as well as decrease tear production during anesthesia [5]. Atropine has been used to prevent bradycardia caused by administration of α_2 -agonists in dogs [6].

Xylazine, an α_2 -agonist used in animal experiments, stimulates α_2 adrenergic receptor in cerebral presynaptic nerve ends, inhibits release of catecholamines and dopamine resulting in analgesic and sedative effects, and hinders nerve conduction in the central nervous system leading to relaxation of striated muscles. Xylazine is usually used in combination with ketamine during anesthetic applications [7].

Diazepam is a potent hypnotic-sedative that causes muscle relaxation. It is a long-acting drug as it is metabolized slowly, and it has relatively weaker cardiovascular effects as compared to other sedative drugs. In combined use with ketamine, diazepam alleviates unwanted cardiovascular effects of ketamine, and demonstrates anticonvulsive, amnestic and muscle relaxant effects [8].

Ketamine increases heart rate and mean arterial pressure, stimulates cardiovascular functions and when used alone. It can induce undesired effects such as muscular hypertonicity, myoclonus, and convulsions. To minimize these unwanted and restricting effects, ketamine is administered in combination with other drug groups such as benzodiazepines, and α_2 -agonists [7,9]. Recently, depending on the species involved, the drug is commonly used in combination

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with diazepam, alpha-2-adrenergic drugs [4]. Since, drugs manifested different effects when they were used separately or in combination, changes that occurred during use of combined-drug should be understood and recognized [7].

The UoG veterinary clinic receives large number of dogs for elective and emergency surgeries. There dogs which are highly aggressive and nervous, even the owner could not restrain them for premedication and general anesthetic administration. Almost all the general anesthetic protocols developed so far for small animal surgery utilizes intravenous administration of drugs. The available protocols are difficult to follow in such highly aggressive and nervous dogs. Hence considering the temperament the dogs, it is decided to induce balanced anesthesia with intramuscular injection of anesthetic agents. Therefore, this study is designed with the objective of developing a general anesthetic protocol suitable for aggressive and nervous dogs, studying the clinical, and hematological changes during general anesthesia and assessing the anesthetic complications during anesthesia and recovery if any.

Materials and Methods

The study was conducted from October 2015 to May 2016 in University of Gondar veterinary clinic, North Gondar, Ethiopia. Bitches that were brought to University of Gondar Veterinary Clinic for elective ovariohysterectomy were used for the study. Based on the physical examination and medical history, apparently healthy bitches alone were selected and the selected bitches (n=12) were randomly divided into two experimental groups using a Randomised Controlled Trials (RCT). All study bitches were assigned into two groups as Group I (with diazepam) and Group II (without diazepam group) each consisting of six based on the anesthetic drugs given; atropine, xylazine, ketamine and diazepam injected groups (G-I) and atropine, xylazine, and ketamine injected groups (G-II).

Assessing physiological parameters

The physiological parameters; (temperature in c_0), heart rate in beats min^{-1} and respiratory rate in breath min^{-1}) were studied before anesthesia, during anesthesia and after recovery. Temperature was taken by inserting a clinical digital thermometer (produce sound after manipulation for 2 minutes in the rectum) at least 1.5-2.0 cm length in to the rectum and keep it in the position. Heart rate and Respiratory rate were recorded by auscultation with a stethoscope place over the left side of chest and counting the abdominal movements respectively [10].

Assessment of hematological parameter

Blood samples (n=3) of 2 ml each was collected aseptically from cephalic vein of each experimental bitch before anesthesia, during anesthesia and after recover. Immediately after collection, the blood samples was transferred in a dry, clean and sterile test tube containing Ethylene Diaminetetra Acetic Acid (EDTA) as anticoagulant. It was used within 2 h after collection to determine Hemoglobin, PCV, TEC and DLC. Hemoglobin (Hb) in gm per dl, Total Erythrocyte Count (TEC) in thousands per cubic millimeters ($10^3/cu\ mm$), the Total Leukocyte Count (TLC) in thousands per cubic millimeters ($10^3/cu$

mm) and Differential Leukocyte Count (DLC) in percentage (%) will be estimated as per standard methods using Automatic Blood Analyzer. The Packed Cell Volume (PCV) in percentage (%) will be estimated by microhaematocrit method. Butter fly catheter (21G and 0.75 inch length) or i.v canula (22G) was fixed in the cephalic vein on the dorsal aspects of the fore limb and taped it to the patient's skin using plaster.

Administration of anesthetic drugs

Drug administration: All bitches were randomly divided into two groups comprising of 6 animals in each group. **Group-I (G-I):** Anesthesia was achieved by administration of atropine (0.04 mg/kg body weight subcutaneously), immediately followed with xylazine-ketamine combination (in the same syringe) (1 mg/kg+10 mg/kg body weight intramuscularly) and after attaining lateral recumbency diazepam (at the rate of 0.5 mg/kg body weight intravenously) was given. **Group-II (G-II):** In this group, anesthesia was achieved by administration of atropine (0.04 mg/kg body weight subcutaneously), immediately followed with xylazine-ketamine combination (in the same syringe) (1 mg/kg+10 mg/kg body weight intramuscularly). The total dose of anesthetic drug required in mg, the total amount of anesthetic drug required in ml and the time of injection for each drug was recorded (Table 1).

The pedal reflex was assessed by deep pricking with a 1.0 inch length 24G hypodermic needle at the coronary band region [10] and the duration loss of pedal reflex was recorded. The total time taken for induction of anesthesia was recorded and calculated by considering time of loss of pedal reflex and time of injection of xylazine-ketamine anesthetics. Incremental dose was considered by adding additional dose of Ketamine @ dose rate of 5.0 mg/kg, i/v was administered if required, till the pedal reflex was completely abolished and the time of 1st and 2nd incremental dose if any was recorded.

Preoperative preparation of animals and ovariohysterectomy surgical procedure

Following anesthesia, all bitches were prepared aseptically for ovariohysterectomy by placing the bitch on surgical table, lying on her back and the surgical teams were prepared for the surgery with a meticulous hand scrubbing, followed by application of a sterile surgical gown and gloves thereby the surgery was started. The site (midline approach) was prepared by shaving or clipping hair aseptically in their respective direction with sterile surgical blade over the middle of the abdomen after scrubbing with surgical soap and the skin is scrubbed with antiseptics (Savlon) to disinfect the area for 5 min. A sterile drape was placed over the surgical site. A scalpel was used to incise the skin at the middle of the abdomen, and then the abdominal cavity was opened. The organs of the female reproductive tracts were identified and the major blood vessels supplying the ovaries and the uterus were ligated (tied off). Sutures (cat gut) that dissolve over time were used to tie off or ligate the blood vessels and also to close the uterus above the cervix. The abdominal incision was then closed with one or two layers of sutures. The outer layer of skin was closed with cotton suture and required to be removed in about 7 days based on clinical union.

Group	Induction of Anesthesia	Maintenance by incremental dose
G-I	a) Atropine 0.04 mg/kg, s.c immediately followed with; b) Xylazine 1 mg/kg, +Ketamine 10.0 mg/kg IM were given. After attaining lateral recumbency Diazepam 0.5 mg/kg, i/v was given	Ketamine 5.0 mg/kg, i/v
G-II	a) Atropine 0.04 mg/kg, s.c immediately followed with, b) Xylazine 1.0 mg/kg, +Ketamine 10.0 mg/kg i.m were given.	Ketamine 5.0 mg/kg, i/v

Table 1: Anesthetic protocol.

Assessment of anesthetic parameters

The anesthetic parameters include quality of induction, duration of anesthesia, complication of anesthesia, time for sternal recumbency, time for unassisted standing, quality and duration of recovery were recorded in all bitches. Type and time of induction of the animals were observed carefully from the moment of premedication thereby administration until the reflexes would be disappeared. Subjective evaluation of induction, muscle relaxation and recovery were monitored. The Induction time, time for sternal recumbency and time for unassisted standing were calculated as per the following guidelines using a stop watch [11].

Induction time and quality: The time interval in minute, between the time of ketamine administration and loss of pedal reflex was taken as induction time and recorded in all the animals. The quality of induction was graded based on scale of 1 (very poor) to 5 (excellent) represented as per the following signs noted in the table [12] (Table 2).

Quality of anesthesia: The quality of anesthesia was assessed based on adequacy of muscle relaxation and presence or absence of salivation, regurgitation and movement in response to surgical incision [13]. The adequacy of muscle relaxation was graded as adequate, if jaws could be opened at ease and abdominal muscles appeared relaxed and inadequate, if difficulty was encountered to open the jaws. The presence of salivation, regurgitation and movement in response to surgical incision, if any was recorded periodically [11].

Duration of anesthesia: The time interval in minute between the injection of anesthetics (time of injection of xylazine+ketamine) to the movement of the first spontaneous elevation of the dogs' head and limb which was recorded in all bitches [7].

Time for sternal recumbency: The time interval in minute, between the time of discontinuation of anesthesia and attainment of sternal recumbency was taken as time for sternal recumbency and was recorded in all the animals [7]. Time for unassisted standing: The time interval in minute between the time of discontinuation of maintenance administration and attainment of unassisted standing was taken as time for unassisted standing and was recorded in all the bitches [11].

Quality of recovery: The quality of anesthetic recovery was assessed and graded as smooth, if no excitement was noticed during recovery and rough if excitement was noticed during recovery.

Duration of recovery: The time interval in minute between the time of movement of the first spontaneous elevation of the dog's head and limb to unassisted standing was taken as time of recovery and was recorded in all bitches [7]. Assessment of post anesthetic complications: The anesthetic complications such as regurgitation, tympany, salivation, post-anesthetic mortality if any, were recorded in all the animals [14].

Data management and analysis

All the data collected during the study period was checked, coded and entered in to Microsoft Excel spreadsheet and analyzed

using SPSS software version 20. Parametric variables were analyzed statistically using Student's independent-samples T-test was used to compare the effect of different treatments at each of the assessment time. Student's independent-samples T-test was used for comparison of mean values for HR, RR, temperature, Hemoglobin, PCV, TEC, TLC, DLC (neutrophil, lymphocyte, monocyte), induction time, duration of anesthesia, time of sternal recumbency, time of unassisted standing and duration of recovery before, during and after anesthesia assessment between two treatment groups. Fisher's exact test (F) was used to compare the incidence of complication during and after anesthesia, quality of induction and quality of recovery. P-value <0.05 (at 5% level of significance) was considered as statically significant. All the data collected was statistically analyzed, compiled and reported.

Results

During the study period, animals remained hemodynamically stable and any problem requiring medical support was not seen. The mean (\pm SD) age of bitches was 2.17 ± 0.835 and the mean (\pm SD) BW of the bitches was 14.75 ± 0.965 . Withholding of feed and water for 12 and 6 hours, respectively prior to induction anesthesia was found to be satisfactory. There was no statistically significant difference ($p > 0.05$) between group I and group II bitches in mean of age and weight such that all the bitches ($n=12$) were found approximately equal weight and age.

Induction of anesthesia

The induction of anesthesia was induced by using atropine (0.04 mg/kg body weight subcutaneously), immediately followed with xylazine-ketamine combination (in the same syringe) (1 mg/kg+10 mg/kg body weight intramuscularly) and after attaining lateral recumbency diazepam (at the rate of 0.5 mg/kg body weight intravenously) for Group I and atropine (0.04 mg/kg body weight subcutaneously), immediately followed with xylazine-ketamine combination (in the same syringe) (1 mg/kg+10 mg/kg body weight intramuscularly) for Group II. The induction doses of the drugs atropine, xylazine and ketamine was not statistically significant ($P > 0.05$) difference between G-I and G-II but diazepam was statistically significant ($P < 0.05$) difference between the two groups.

Quality of induction: The quality of induction was assessed and graded as excellent, good, fair, poor and very poor. There was statistically significant difference ($P < 0.05$) in quality of induction between group I and group II bitches. The quality of induction was excellent in 4 bitches from G-I but no from G-II, good in 2 bitches from G-I and G-II, fair and poor in 3 bitches and 1 bitch from G-II respectively. No bitch was found fair, poor or very poor induction from G-I.

Quality of anesthesia: The quality of anesthesia was assessed based on adequacy and inadequacy of jaw and abdominal muscle relaxation and presence or absence of salivation, regurgitation, urination, defecation and movement in response to pain stimuli. There was statistically significant difference ($P < 0.05$) in muscle relaxation between group I and group II bitches. The abdominal muscle and jaw muscle relaxation was adequate in 5 bitches and two bitches from G-I

	Score	Description/sign
Induction	1	Very poor, unpredictable fall
	2	Poor, attaining recumbency unpredictably, but no injuries
	3	Fair, Bitches slowly attains sternal or lateral recumbency, marked paddling of limbs or shaking of head
	4	Good, slowly attains recumbency, only slight paddling of limbs or shaking of the head
	5	Excellent, recumbency achieved slowly and smoothly, no paddling or head shaking

Table 2: Quality of induction.

and G-II respectively. However, the abdominal muscle and jaw muscle relaxation was also found inadequate in 1 bitch and 4 bitches from G-I and G-II bitches respectively. Salivation and vomiting were not statistically significant ($P>0.05$) difference between G-I and G-II in which salivation was encountered in 1 bitch from G-II and vomiting was encountered in 1 bitch from G-I during anesthesia. Regurgitation and urination during anesthesia were not also found statistically significant ($P>0.005$) between both groups but here was statistically significant ($P<0.05$) difference in response to surgical incision in response to pain stimuli between groups. Response to surgical incision was observed in 3 bitches from G-II but not observed in all bitches from G-I ($n=6$).

Anesthetic parameter

The anesthetic parameters: induction time, duration of anesthesia, time of return of pedal reflex, time of head right reflex, time for sternal recumbency, time for unassisted standing in minutes recorded during the study were presented in Table 3.

Physiological parameters

The mean values of HR, RR and rectal temperature were differing before, during, after anesthesia. All parameter was assessed before during and after anesthesia and was not significantly decreased in group I bitches ($P>0.05$). The data for HR, RR and rectal temperature before, during and after anesthesia were summarized in Table 3 (Figure 1).

Hematological parameters

Hb, PCV, TEC, TLC and DLC were recorded and analyzed before anesthesia, during surgery and after recovery. Comparison of the mean (\pm SD) of Hb, PCV, TEC, TLC and DLC among the two groups revealed that there were significant differences ($P<0.05$) in PCV during anesthesia between group I and group II but no significance difference ($p>0.05$) were recorded in the other hematological parameters. The data were summarized in Figure 2.

Data on nature of recovery

The quality of recovery was assessed and graded as smooth and rough. The quality of recovery was found statistically significant ($P<0.05$) difference between group I and Group I. The quality of recovery was found almost smooth in G-I when comparison was considered with G-II in which all were appearing calm. However, during working time G-II bitches were experienced as transient excitement, struggle to stand, and moderate ataxia in which recovery was almost rough as compared with G-I.

Timing of anesthesia	Group	N	Mean \pm Std. Dev	P-value
Induction of time	I	6	6.00 \pm 0.894	0.000
	II	6	9.67 \pm 1.211	
Time of loss of pedal reflex	I	6	3.00 \pm 0.000	0.003
	II	6	5.00 \pm 0.894	
Duration of anesthesia	I	6	67.83 \pm 2.994	0.000
	II	6	59.17 \pm 1.941	
Time of sternal recumbency	I	6	12.17 \pm 2.483	0.002
	II	6	7.17 \pm 1.47	
Time of unassisted standing	I	6	11.83 \pm 2.483	0.001
	II	6	6.33 \pm 1.033	
Time for duration of recovery	I	6	17.67 \pm 2.251	0.004
	II	6	13.83 \pm 1.169	

Std. Dev.=Standard Deviations; Mean difference is significant ($P<0.05$); N=number of animals

Table 3: Mean (\pm SD) of Anesthetic parameter.

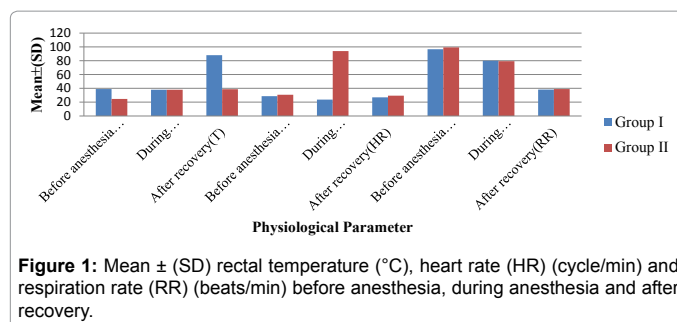


Figure 1: Mean \pm (SD) rectal temperature ($^{\circ}$ C), heart rate (HR) (cycle/min) and respiration rate (RR) (beats/min) before anesthesia, during anesthesia and after recovery.

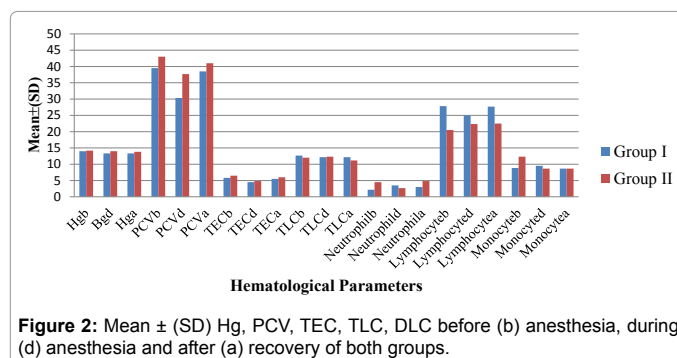


Figure 2: Mean \pm (SD) Hg, PCV, TEC, TLC, DLC before (b) anesthesia, during (d) anesthesia and after (a) recovery of both groups.

Post anesthetic complication

Post anesthetic complications were assessed whether salivation, regurgitation, urination or mortality was encountered after recovery in both groups. Salivation was found statistically significant ($P<0.05$) in group I bitches as compared to Group II in which salivation was encountered in 5 bitches from G-I but 1 bitch from G-II. Regurgitation was not encountered at all in both groups. Urination was present in 1 bitches from G-I which was not significant ($P>0.05$). All bitches ($n=12$) were survived the surgery and recovered from anesthesia without any death.

Discussion

Various sedatives and tranquilizing agents are used as pain killers and/or chemical restraints while animals undergo minor or major surgeries. These drugs are needed in veterinary practice and are indispensable as they help in overcoming resistance of the animals during examination, maintaining depth of anesthesia, reducing the amount of anesthetic agents and increasing margins of safety [3]. The purpose of anesthesia is to provide reversible unconsciousness, amnesia, analgesia, and immobility with minimal risk to the patient [1].

Although the sedative and hemodynamic effect of diazepam has been reported previously, the sedative effect of simultaneous administration of diazepam and xylazine-ketamine in dogs has not been studied to current study knowledge. In present study, 12 indigenous bitches are taken with approximately equal age and body weight 2.17 ± 0.835 and 14.75 ± 0.965 respectively when comparison was considered. OHE was done using xylazine- ketamine with diazepam for Group I and xylazine- ketamine without diazepam for Group II provided that atropine was given for both groups as a premedication. This is in line of agreement with several studies using this mixture for general anesthesia in dog's anesthetic drug [15]. OHE was performed by xylazine- ketamine anesthesia with and without diazepam and this agreed with Dellinger [16] and William et al. [2].

In present study, xylazine-ketamine combination and diazepam is given as single IM and IV injections respectively in order to

avoid distress caused by multiple injections. In the clinical setting, intramuscular injection is the preferred route of drug administration for sedation and premedication because minimal restraint is required. Besides, cardiovascular responses are attenuated when anticholinergics and α 2-agonists are administered intramuscularly and when used in combination, the adverse actions of both drugs may be diminished due to the lower doses required [15].

The induction of anesthesia produced by G I was found fast, recumbency achieved slowly and smoothly as compared to G II. This result of the present study was in line with the findings of Azizpour and Hassani [12]. The fast induction of anesthesia result in G I was in contrary to the reports of the earlier studies in sheep [7] but because in the current study, there was no significant difference in age species and breed. This study was also investigated that loss of pedal reflex and duration of induction which was shown in Table 3 was significantly reduced. Because when diazepam and ketamine used together, they have a synergistic effect resulting in a smooth recovery and better muscle relaxation and their efficacy is enhanced whilst minimising their unwanted adverse effects which was in line of agreement with Sumitra et al. [17] conducted a research on male Wistar rats.

In this study, muscle relaxation was significantly ($p < 0.05$) adequate in the G I than G II. Improvement of muscular relaxation in G I was associated with the muscle relaxant properties of diazepam. Diazepam has potent muscle relaxant and anticonvulsant properties and has been used in a wide range of wild and domestic animals and birds [18]. Jaw muscle relaxation was significantly ($P < 0.05$) adequate in G I which was in accordance with earlier studies where resistance to open the mouth fully is lost in moderate anesthesia, hence jaw tone is considered to be a useful indicator of anesthesia [19,20]. In the present study, salivation, urination and vomiting was not a significant difference between the two groups before anesthesia, during anesthesia and after anesthesia. This result demonstrated in accordance with the previous reports in which it has been demonstrated that anti-muscarinic drugs like atropine has decreasing effect on hyper secretion induced with ketamine [5,21]. In the present study, atropine was also incorporated in both groups as premedication which decreased hyper secretion which was induced during spontaneous breathing.

In the current study, duration of anesthesia for G I was found significantly longer as compare to G II which was in accordance with the previous studies where the highest duration of anesthesia was observed in the study of birds [8]. This might be due to wide-distribution of diazepam in the body, as because diazepam is highly soluble in lipid and can be redistributed into muscles and adipose tissues [22]. Furthermore, diazepam is a long-acting drug due to its slow-metabolism in the body as compared to the other sedatives [23]. The results obtained here were in line with the findings of Azizpour and Hassani [12] who performed general anesthesia in pigeons using a combination of ketamine HCL and diazepam. Lin et al. [24] investigated the effects of two different anesthetic regimens, namely ketamine-diazepam and ketamine- diazepam-xylazine combinations in sheep and found that the latter combination resulted in a longer duration of anesthesia.

In the present study, smooth recoveries were observed significantly in G I than G II which was in line with finding of Durrani et al. [8] and Mahmud et al. [4] that smooth recoveries were observed in diazepam-ketamine anesthesia. The longest recovery period was observed in the birds of diazepam-ketamine anesthesia, which was desirable since diazepam could augment ketamine's anesthetic effects decreasing its side effects; thus, it provided necessary depth and duration

of anesthesia for the comfortable completion of surgeries [4,25]. Ketamine and diazepam induced a synergistic action producing a deep analgesia for long duration. Similar observations were also reported in pigeons using ketamine-diazepam [8,18]. This result is in line with the present study in which the duration of recovery of G I was significantly longer than G II.

Ketamine, in contrast to most of the anesthetic drugs, it has been shown to possess incremental effects on the heart rate, blood pressure and respiratory rate due to increase in sympathetic activation. It had also unwanted respiratory effects such as increase in respiratory secretions [21]. Diazepam decrease cardiovascular effects of ketamine [9]. Therefore the unwanted anesthetic effect of ketamine was compensated and balanced by xylazine and diazepam through lowering blood pressure and decreasing heart and respiratory rate.

In the current study, the mean \pm (SD) of temperature, respiratory rate and heart rate of both groups before anesthesia, during anesthesia and after anesthesia were considered and compared between the groups. There were decrements of all the three parameters during anesthesia and came to the normal physiological range after recovery but the decrease in the physiological parameter was not found statistically significant ($P > 0.05$) between the groups. These results were in accordance with previous studies as Coulson et al. [26] investigated cardiovascular effects of ketamine-diazepam in sheep and stressed lack of any meaningful effects on heart rates and respiratory rate. In this study, alterations in physiological parameter caused by both drug combinations remained within physiological limits.

Demirkan et al. [27] and Atalan et al. [15] emphasized that rectal temperature decreased during the anesthesia. Anesthetic combination decreased significantly the mean rectal temperature during surgery and the decline was highly significant after surgery. However, in the present study of the mean \pm (SD) of rectal temperature of both groups began to decrease during anesthesia but came to the physiological range after recovery and the difference between the groups was not significant ($P > 0.05$). This finding is in agreement with previous reports [28].

The mean \pm (SD) of the Haemoglobin, PCV, TEC, TLC and DLC were assessed before, during and after anesthesia and were slightly decreased for a short time in both groups during anesthesia but the alteration was not significant ($P > 0.05$) between the groups. However, the values for PCV was significantly decreased ($P < 0.05$) and the result was in accordance with previous studies [29,30]. Pooling of circulating blood cells in the spleen and other reservoirs secondary to decreased sympathetic activity could be the reason for a decrease in PCV. The decrease in PCV during the period of anaesthesia or sedation might be attributed to the shifting of fluid from extravascular compartment to intravascular compartment in order to maintain normal cardiac output in the animals [29].

The occurrence of post anesthetic complication in both groups were assessed and it was found not statistically significant ($P > 0.05$) except salivation which was more experienced in G I than G II which is supported by previous reports [31]. Although diazepam is an initial cause of salivation, but the loss of spontaneous swallowing and the loss of spontaneous tongue reflex occurred during this study might be the cause of salivation observed after recovery as a result of diazepam injection.

Conclusion

The study was conducted on 12 female apparently healthy indigenous bitches which were randomly grouped in to Group I and Group II. Anesthesia was induced using atropine (0.04 mg/kg

BW, S.C) immediately followed with xylazine-ketamine (1.0 mg /kg/ BW+10.0 mg/kg BW, I.M) and after lateral recumbency, diazepam (0.5 mg /kg BW, i/v) was administered for G-I and atropine (0.04 mg/kg BW, S.C) immediately followed with xylazine-ketamine (1.0 mg /kg/ BW+10.0 mg/kg BW, I.M) for G-II. The anesthetic parameters; quality of induction, time loss for pedal reflex, induction time, duration of anesthesia, quality of anesthesia, time for sternal recumbency, time for unassisted standing, duration and quality of recovery were recorded and analyzed on both Group I and Group II and all the parameters were found statistically significant. The results of the present study concluded that atropine-xylazine-ketamine-diazepam combination is useful anesthetic protocol for excellent induction, adequate muscle relaxation, satisfactory duration of anesthesia and smooth recovery for OHE in bitches although it might result in prolonged recovery and some complication like salivation rarely as compared to atropine-xylazine-ketamine combination. Both drug combinations do not affect the physiological and hematological parameters of the animals during study time and both of them can be safe for OHE if used safely and appropriately. However, further studies are required to evaluate the effects on cardiopulmonary function of these drug combinations in detail.

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