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Combined Effect of Ketamine with Diazepam and Ketamine with Midazolam for Neutering in a Dog Model

[*1]Ghulam Mujtaba Gadani, [*2]Ahmed Nawaz Tunio, [3]Sadam Hussain Gadani, [4]Abdul Salam Khoso, [5]Abdul Manan Chohan

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Ghulam Mujtaba Gadani
Department of Surgery and Obstetrics, Sindh Agriculture University, Tandojam, Pakistan
Ahmed Nawaz Tunio*
Department of Surgery and Obstetrics, Sindh Agriculture University, Tandojam, Pakistan. Corresponding Author Email: antunio@sau.edu.pk
Sadam Hussain Gadani
Department of Animal Reproduction, Sindh Agriculture University, Tandojam, Pakistan
Shahzeb Arain
Department of Veterinary Microbiology, Sindh Agriculture University, Tandojam, Pakistan
Abdul Salam Khoso
Department of Surgery and Obstetrics, Sindh Agriculture University, Tandojam, Pakistan
Abdul Manan Chohan
Department of Animal Reproduction, Sindh Agriculture University, Tandojam, Pakistan

ABSTRACT

Neutering is a common surgical technique performed in animals to remove the testicles. In dogs, it is performed to control the population to reduce the risk of rabies. Neutering can be performed under general anesthesia on a dog, and it is the most painful and requires an ideal anesthetic combination that produces better analgesia, anesthetic effect, and muscle relaxation. Anesthesia is produced by drugs that decrease the activity of nervous tissue locally, regionally, or within the central nervous system. The combined use of ketamine with diazepam provides balanced anesthesia, which induces analgesia and unconsciousness, and midazolam is used in combination with ketamine for muscle relaxation and intramuscular absorption. In this study, 8 healthy male dogs were divided into two groups, A and B. Dogs in group A were anesthetized with ketamine and diazepam; however, in group B, the combination of ketamine and midazolam was administered. Results show that the onset of anesthesia was 8.01±0.41 and 6.5±0.29 minutes in groups A and B, respectively. The mean duration of anesthesia in both groups was 33.5±0.65 minutes in group A and 27.8±0.85 minutes in group B. The degree of anesthesia and analgesia was 2.65±0.36 and 2.54±0.05 in both groups; however, the degree of analgesia was 2.65±0.36 and 2.54±0.05, respectively. The recovery time in groups A and B was 61.01±1.29 and 51.50±1.44 minutes, respectively. Rectal temperature of dogs was 99.74±0.19 and 100.44±0.40 in groups A and B, respectively. The heart rate of dogs was 82.27±0.79 and 82.19±0.55 in groups A and B, respectively. The respiratory rate was 20.23±0.34 and 21.23±0.92 in groups A and B. It is concluded that both groups produced better anesthesia for neutering, but ketamine+midazolam had some disadvantages, such as urination and excessive salivation in dogs.

INTRODUCTION

Neutering is a common surgical technique performed in animals to remove the testicles. In dogs, it is performed to control the population to reduce the risk of rabies. Overpopulation of stray dogs is a worldwide problem that compromises public health and animal welfare and increases the risk of rabies in humans and animals, which changes the animal behavior and leads to death (Puri et al., 2018; Soto et al., 2018). Neutering reduces the hormonal levels in the body that control reproduction and sexual behavior in male animals, influencing physical and behavioral characteristics. Surgically removing the testicles in male dogs and cats is a common method of neutering (Mckenzie, 2010; Guerrero et al., 2016). Neutering can be performed under general anesthesia on a dog, and it is the most painful and requires an ideal anesthetic combination that produces good analgesia, anesthetic effect, and muscle relaxation. An ideal anesthetic protocol is characterized by a rapid onset and smooth recovery, producing unconsciousness, muscle relaxation, and analgesia while maintaining cardiovascular and respiratory function. Anesthesia is produced by drugs that decrease the activity of nervous tissue locally, regionally, or within the central nervous system (Bhave et al., 2019). Ketamine increases heart rate and stimulates cardiovascular function when used alone. It can induce undesired effects such as stiffness of muscles, involuntary muscle jerking, and involuntary muscle contraction. To minimize the unwanted and restricting effects, ketamine is administered in combination with other drug groups such as benzodiazepines (Tranquilli and Grimm, 2015; Arain et al., 2025). Diazepam is a potent hypnotic-sedative that causes muscle relaxation. It is a long-acting drug that is metabolized slowly and has relatively few cardiovascular effects compared to other sedative drugs. The combined use of ketamine with diazepam provides balanced anesthesia, which induces analgesia and unconsciousness (Nesgash et al., 2016; Ferreira et al., 2015).

Diazepam alleviates unwanted cardiovascular effects of ketamine and demonstrates anticonvulsive, amnestic, and muscle relaxant effects. The combination of diazepam and ketamine is a commonly used protocol for the induction of general anesthesia in dogs of various ages. It is particularly useful in certain cases with cardiovascular compromise (Shahzeb et al., 2023; Uludag et al., 2020). The combination is generally associated with smooth, excitement-free induction of anesthesia. Recovery from diazepam and ketamine anesthesia in dogs is free from emergence excitation, although ataxia is commonly observed. However, midazolam is a short-acting hypnotic-sedative benzodiazepine with anxiolytic, anticonvulsant, hypnotic, and amnesic properties (Bellini et al., 2014; Arain et al.,

2024). Midazolam is highly advantageous due to its rapid onset of action (1-2 minutes when administered intravenously) and its short recovery period (30-60 minutes). It is also administered to prevent the side effects of ketamine. Midazolam is used in combination with ketamine due to its muscle relaxant properties and its fewer cardiovascular effects, as well as its intramuscular absorption. Sedation obtained by combining ketamine and midazolam produces muscle relaxation. When ketamine is combined with diazepam, it produces a long and safe anesthetic effect (Yohannes et al., 2018). To minimize side effects and improve recovery from anesthesia, this study was designed. The objective was to compare the anesthetic, clinical, and physiological changes, as well as assess anesthetic complications, of ketamine combined with diazepam versus ketamine combined with midazolam for neutering in dogs.

MATERIALS AND METHODS

ETHICAL STATEMENT

The protocols applied in this study were approved by the Research Ethics Committee of the Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam, Pakistan (Approval No. DAS-425-2019).

EXPERIMENTAL ANIMALS

This study was conducted on eight healthy adult male dogs. The dogs were randomly divided into two groups: Group A (n=4) and Group B (n=4). The body weight and age of the experimental dogs are shown in Table 1.

TABLE 1: EXPERIMENTAL ANIMALS

Animal Tagging No	Body weight (Kg)	Age (Months)
1.	14	12
2.	16	13
3.	15	12
4.	13	12
5.	16	14
6.	14	12
7.	12	10

8.	14	11

PRE-ANESTHETIC MEDICATION

Atropine sulphate (Selmore Pharma) was administered at a dose rate of 0.04mg/kg body weight 15 minutes before induction of anesthesia.

INDUCTION OF ANESTHESIA

The dogs were anesthetized with ketamine (Neutro Pharma) and diazepam (Mediate Pharmaceutical). In group B, dogs were anesthetized with a combination of ketamine and midazolam (Indus Pharma). Both injectable anesthetic drugs were administered using a 3ml disposable syringe with a 24-gauge needle.

TABLE 2: EXPERIMENTAL DESIGN

Groups	No. of Animals	Route of Administration	Treatment	Dose (mg/kg)
A	04	Intramuscular	ketamine+diazepam	10mg/kg,0.5mg/kg
B	04	Intramuscular	ketamine+midazolam	10mg/kg,0.5mg/kg

SURGICAL PROCEDURE FOR CASTRATION

The anesthetized dogs were placed in dorsal recumbency, and the surgical site was scrubbed with disinfectant solutions (hydrogen peroxide and Povidone-iodine). Hair clipping was performed with the help of an Oster A5 hair clipper, and the surgical site was draped with a laparotomy sheet. For castration, a 2 to 3-cm-long single incision was made on the scrotum, just anterior to the testicles. Then internal layers, including the tunica dartos and tunica vaginals, were incised to visualize the testicles. Each testicle was separated from the epididymis. The spermatic artery and vein were ligated using chromic catgut 2-0 (Ethos Healthcare) to control bleeding. After ligation, the testicles were removed with the help of a surgical blade. The spermatic vessels were placed under the inner layer, and then the layers were sutured with chromic catgut (2-0). The skin wound was closed with silk using a simple interrupted suture pattern. The operated dogs were monitored during anesthesia until full recovery.

POST-OPERATIVE CARE

After suturing, povidone was applied to the wound, and amoxicillin (10 mg/kg body weight) was administered for five days to prevent the secondary infection, and diclofenac sodium (Sami Pharma),

along with meloxicam (Selmore Pharma), purchased from the vet store, was administered to reduce pain and inflammation. Semi-solid diet was provided until complete healing was achieved.

EFFECT OF ANESTHESIA

The dogs were monitored during the whole experiment to record the various parameters like onset of anesthesia, duration of anesthesia, degree of anesthesia, analgesia and recovery from the anesthesia along with physiological parameters rectal temperature, pulse rate (beat per minute), respiratory rate (breath per minute) and clinical signs also be observed such as, recumbancy, salivation, frequent urination, anal relaxation, corneal reflex, pedal reflex, jaw tone reflex and tongue pinch reflex.

STATISTICAL ANALYSIS

The statistical analysis was performed to evaluate the effect of the factor (group) on study parameters. Analysis of variance (ANOVA) and the least significant difference (LSD) test were used to compare means and percentages, and differences were considered significant at ($P< 0.05$).

RESULT

ONSET, DURATION, AND DEGREE OF ANESTHESIA, DEGREE OF ANALGESIA, AND RECOVERY PERIOD DURING CASTRATION IN DOGS

Table 1 indicates that the onset of anesthesia in group A (ketamine +diazepam) was achieved within 8.01±0.41 minutes after intramuscular administration. At 6.5±0.29 minutes, group B (ketamine + midazolam) exhibited a significantly ($P<0.05$) faster onset of anesthesia than group A, indicating that induction was delayed in group A. The duration of anesthesia in group A was 33.5 ± 0.65 minutes, while in group B, it was 27.8 ± 0.85 minutes. The duration of anesthesia is longer in group A as compared to group B. The degree of anesthesia after the administration of ketamine + diazepam in group A was surgical anesthesia lasting from 25 to 45 minutes, while in group B, the administration of ketamine + midazolam produced surgical anesthesia lasting from 20 to 40 minutes; both groups showed better analgesia during anesthesia. It was noticed that ketamine + diazepam produces better analgesia than ketamine combined with midazolam. The recovery from anesthesia was safe in both treatments. No completion was observed, such as cardiac arrest, respiratory depression, excitement, respiratory arrest, or nerve paralysis after anesthesia. The recovery time for the ketamine+diazepam and ketamine+midazolam was 61.01±1.29 and 51.50±1.44 minutes, respectively. A significant ($P<0.01$) difference was observed between the two anesthetic combinations, with the recovery duration being shorter in ketamine+midazolam compared to ketamine+diazepam.

TABLE 1: ONSET, DURATION, AND DEGREE OF ANESTHESIA, DEGREE, AND RECOVERY PERIOD DURING CASTRATION IN DOGS

S.no	Parameters	Group A Ketamine+Diazepam (Mean±SE)	Group B Ketamine+Midazolam (Mean±SE)	P-values	Significance
1	Onset of anesthesia	8.01±0.41	6.5±0.29	0.027	* (P<0.05)
2	Duration of anesthesia	33.5±0.65	27.8±0.85	0.002	**(P<0.01)
3	Degree of anesthesia	2.65±0.36	2.54±0.05	0.781	ns
4	Degree of analgesia	2.65±0.36	2.54±0.05	0.781	ns
5	Recovery period	61.01±1.29	51.50±1.44	0.0028	**(P<0.01)

Values are expressed as mean ± SE. ns = not significant (P>0.05), * = P<0.05, ** = P<0.01.

RECTAL TEMPERATURE, PULSE RATE, AND RESPIRATORY RATE DURING CASTRATION IN DOGS

Table 2 shows that the mean values for rectal temperature of dogs were 99.74±0.19 °C in group A, and 100.27±0.40 °C in group B. After induction of anesthesia, rectal temperature decreased slightly in both groups; however, there was no significant (P>0.05) difference between groups. The mean values of pulse rate in dogs were recorded as 82.27±0.79 in group A, and 82.19±0.55 in group B, respectively. The mean pulse rate of dogs was 82.27 ± 0.79 in group A and 82.19 ± 0.55 in group B, with no significant (P>0.05) difference observed between groups. The mean respiratory rate was 20.23 ± 0.34 in group A and 21.23 ± 0.92 in group B. Although both groups showed a mild decrease in respiratory rate after induction, the difference between groups was not statistically significant (P>0.05).

TABLE 4: MEAN VALUES FOR THE RECTAL TEMPERATURE, PULSE RATE, AND RESPIRATORY RATE DURING CASTRATION IN DOGS

S. no	Parameters	Group A Ketamine+Diazepam (Mean±SE)	Group B Ketamine+Midazolam (Mean±SE)	P-values	Significance
1	Rectal temperature (°C)	99.74±0.19	100.44±0.40	0.184	ns
2	Pulse rate (PR)	82.27±0.79	82.19±0.55	0.937	ns
3	Respiratory rate (RR min^{-1})	20.23±0.34	21.23±0.92	0.368	ns

Values are presented as mean ± SE. ns = not significant (P > 0.05).

OBSERVATION OF REFLEXES DURING CASTRATION IN DOGS

Table 4 shows that the observation of reflexes after induction of anesthesia in castrated. Reflexes observed included salivation, urination, anal relaxation, analgesia, corneal reflexes, pedal reflexes, jaw tone reflexes, and tongue pinch. Recumbency and salivation were present in groups A and B. Frequent urination was absent in group A, but present in group B, while anal relaxation was present in both groups. Corneal, pedal, jaw tone, and tongue pinch reflexes were absent in group A and group B.

TABLE 5: REFLEXES AFTER INDUCTION OF ANESTHESIA IN GROUPS A AND B DURING CASTRATION IN DOGS

Reflexes	Treatment	
	Group A	Group B
Recumbency	Present	Present
Salivation	Present	Present
Frequent urination	Absent	Present
Anal relaxation	Present	Present
Corneal reflex	Absent	Absent

Pedal reflex	Absent	Absent
Jaw tone reflex	Absent	Absent
Tongue pinch reflex	Absent	Absent

DISCUSSION

The present study showed that the onset of anesthesia was achieved within minutes in both groups. Induction was slightly delayed in group A (ketamine + diazepam) compared with group B (ketamine + midazolam). Similar findings were reported by Youhnnes et al. (2018), who observed a slower onset of anesthesia with ketamine + diazepam. Bellini et al. (2014) also reported significant differences ($p<0.05$) in induction times with different anesthetic combinations in rabbits. The duration of anesthesia was longer in group A than in group B. Iqbal et al. (2020) similarly reported a prolonged anesthetic duration with ketamine + diazepam. In the present study, both groups achieved surgical anesthesia (stage III, plane 3), lasting approximately 25-45 minutes in group A and 20-40 minutes in group B, consistent with the findings of Ozkan et al. (2010). Bellini et al. (2014) also noted that both ketamine + diazepam and ketamine + midazolam produced adequate surgical anesthesia, though with variation in duration. Analgesia was effective in both groups. The present findings align with those of Bellini et al. (2014) and Ozkan et al. (2010), who reported that ketamine combined with diazepam generally provides superior analgesia compared with ketamine combined with midazolam.

In this study, the degree of analgesia appeared slightly deeper in group A, which is consistent with reports that ketamine + diazepam has a stronger analgesic effect, as observed in sheep by Ozkan et al. (2010). Recovery from anesthesia was smooth and uneventful in both groups. Recovery time was significantly longer ($p<0.05$) in group A compared with group B. These findings are consistent with those of Durrani et al. (2008) and Sumitra et al. (2004), who reported delayed recovery with ketamine + diazepam compared with ketamine + midazolam. Rectal temperature decreased slightly in both groups after induction, although the difference between groups was not statistically significant ($p > 0.05$). Hansen (2003), Iqbal et al. (2020), and Mahmud et al. (2014) similarly observed reductions in rectal temperature following the administration of ketamine and diazepam. Pulse rate was comparable between the two groups, with no significant difference ($p > 0.05$). These findings agree with Hansen (2003), who reported initial bradycardia followed by a compensatory increase with ketamine + diazepam, and Iqbal et al. (2020), who observed reduced cardiac output. In the present study, ketamine + midazolam produced a gradual decrease in heart rate within the first 10 minutes, followed by a

return toward baseline, consistent with the observations of Uludag et al. (2020) and Lopes et al. (2017). Kropf and Hughes (2018) reported similar patterns in goats, with ketamine + midazolam increasing heart rate after 30 minutes. Respiratory rate decreased in both groups after induction. The minimum rate was observed at 10 minutes in group A and at 5 minutes in group B, after which it gradually increased but remained below baseline during anesthesia. These results are comparable with those of Hansen (2003), Iqbal et al. (2020), and Bellini et al. (2014), who also reported respiratory depression with these anesthetic combinations. Brodbelt et al. (2008) and Mahmud et al. (2014) similarly found that ketamine + midazolam produced a transient decrease in respiratory rate.

The observation of reflexes revealed that recumbency and anal relaxation were consistently present in all dogs from both groups. Corneal, pedal, jaw tone, and tongue pinch reflexes were uniformly absent, indicating that both ketamine + diazepam and ketamine + midazolam combinations provided sufficient depth of anesthesia. Salivation was observed in both groups, whereas frequent urination was noted only in the ketamine + midazolam group. These findings agree with Raekallio et al. (2002), who reported that the ketamine + diazepam combination resulted in better control of reflexes compared with ketamine + midazolam. Similarly, Dupras et al. (2001) observed reflex depression in equines with both drug combinations, with ketamine + diazepam providing greater suppression.

CONCLUSION

It was concluded from the present study that the effect of ketamine+diazepam and ketamine+midazolam produced better anesthesia and analgesia during neutering in dogs. It was noticed that ketamine+midazolam showed some disadvantages, like frequent urination and excessive salivation in dogs.

CONFLICT OF INTEREST

The authors do not have any conflicts of interest.

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