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Effect of Increased Dose of Dexmedetomidine *Vis-à-vis* Addition of Fentanyl on Clinical and Cardio-respiratory Actions of Dexmedetomidine-midazolam-ketamine Anaesthesia in Dogs

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ABSTRACT

The present study was designed to compare the influence of increased dose of dexmedetomidine and addition of fentanyl on the course of anaesthesia produced by dexmedetomidine-midazolam-ketamine combination in dogs. A prospective randomized blinded study was conducted on 12 client owned adult mixed breed dogs of either sex, divided into three groups. In the animals of group A, dexmedetomidine $10 \mu\text{g kg}^{-1}$ b.wt. and midazolam 0.4 mg kg^{-1} were administered simultaneously in the thigh muscles. In group B, dexmedetomidine $20 \mu\text{g kg}^{-1}$ and midazolam 0.4 mg kg^{-1} were administered. In the animals of group C dexmedetomidine $10 \mu\text{g kg}^{-1}$, midazolam 0.4 mg kg^{-1} and fentanyl $4 \mu\text{g kg}^{-1}$ were administered. Ten minutes later, ketamine was administered I.V. in all the groups to induce anaesthesia. Excellent muscle relaxation was observed up to 30 min in groups A and B and up to 75 min in group C. Pedal reflexes were abolished up to 45 min in groups A and B and up to 75 min in group C. Only 25% animals in group A permitted intubation, but intubation could be performed in all the animals of groups B and C. Weak time and down time were significantly ($p < 0.05$) shorter in group B than in group A. Sternal recumbency time was significantly ($p < 0.05$) longer in group B as compared to groups A and C but standing recovery time was significantly ($p < 0.05$) shorter in group C as compared to groups A and B. Heart rate decreased significantly ($p < 0.05$) in groups B and C but MAP (Mean Arterial Pressure) remained unchanged. In group A heart rate did not change significantly but MAP showed significant ($p < 0.01$) decrease. Respiratory rate decreased significantly ($p < 0.05$) in groups A and B but SPO_2 (Saturated Oxygen) was maintained near the base line in all the groups. It was concluded that dexmedetomidine ($10 \mu\text{g kg}^{-1}$) -midazolam (0.4 mg kg^{-1})-ketamine combination produced anaesthesia for 45 min. Increasing the dose of dexmedetomidine to $20 \mu\text{g kg}^{-1}$ did not offer any advantage, however, addition of fentanyl ($4 \mu\text{g kg}^{-1}$) not only increased the duration of anaesthesia up to 60 min but also facilitated the recovery without additional adverse side effects.

Key words: Dexmedetomidine, dogs, fentanyl, ketamine, midazolam

INTRODUCTION

Dexmedetomidine is the latest alpha-2 agonist with very high specificity and highest potency among the drugs of this group. Dexmedetomidine is the active enantiomer of the racemate medetomidine and when administered at half the dose, induces similar effects as medetomidine

(Ansah *et al.*, 1998). However, its use is characterized by marked cardiovascular effects, classically described as bradycardia, increased systemic vascular resistance, decreased cardiac index and increased central venous pressure (Bloor *et al.*, 1992). To offset these effects combinations of dexmedetomidine have been proposed with other sedatives and analgesics (Leppanen *et al.*, 2006) and even local anaesthetics (Ahmed *et al.*, 2008; Abosedira, 2008).

Midazolam, a benzodiazepine derivative is used as premedicant, sedative and an anesthetic induction agent. Administration of midazolam is associated with modulatory influences on postoperative pain mechanisms (Ali and Maryam, 2007). It has a short duration of action, with a rapid elimination half life and a total body clearance. It has minimal effect on cardiac function (Butola and Singh, 2007). Respiratory rate depression due to midazolam is not associated with hypoxia (Fakheri *et al.*, 2010).

Opioid agonists are reported to have synergistic interactions with α -2 agonists and benzodiazepines. Fentanyl citrate is potent narcotic analgesic notable for its rapid onset and brief duration of action (Corssen *et al.*, 1964) and is widely used for surgical analgesia and sedation (Huq, 2007). It is 50-100 times more potent than morphine (Thurmon *et al.*, 1999). Fentanyl in therapeutic doses produces little effect on cardiac output and blood pressure (Thurmon *et al.*, 1999).

Dexmedetomidine has been used as a premedicant in laboratory dogs through IV route (Kuusela *et al.*, 2001) which is less preferred as compared to IM route in veterinary practice. Intra-muscular route has been evaluated along with other sedative/analgesics for the purpose of sedation without induction of general anaesthesia (Leppanen *et al.*, 2006; Ahmad *et al.*, 2011). Dexmedetomidine has been reported to have a ceiling of effects (Kuusela, 2004).

The purpose of the paper was to study the effects of increasing the dose *vis-à-vis* addition of fentanyl on the quality of sedation, cardiovascular changes and ketamine-sparing ability of 10 $\mu\text{g kg}^{-1}$ dexmedetomidine in dogs premedicated with midazolam.

MATERIALS AND METHODS

A prospective randomized blinded study was conducted on 12 client owned, mixed breed adult dogs of either sex. The animals were deemed healthy through physical examination and divided randomly into three equal groups, A, B and group C. In the animals of group A, dexmedetomidine 10 $\mu\text{g kg}^{-1}$ b.wt. (Dexdomitor; Orion Pharma, Finland) and midazolam 0.4 mg kg^{-1} (Mezolan; Neon Laboratories, Palghar, India) were administered simultaneously in the thigh muscles. Ten minutes later, ketamine (Ketamin, Themis Medicare, Uttarakhand, India) was administered I.V. to the effect. In the animals of group B, dexmedetomidine 20 $\mu\text{g kg}^{-1}$ and midazolam 0.4 mg kg^{-1} were administered in the thigh muscles followed, 10 min later, by ketamine iv until effect. In the animals of group C dexmedetomidine 10 $\mu\text{g kg}^{-1}$, midazolam 0.4 mg kg^{-1} and fentanyl 4 $\mu\text{g kg}^{-1}$ (Fendrop; Sun Pharmaceutical India Ltd.) were administered simultaneously in the thigh muscles and 10 min later, ketamine was administered I.V. to the effect.

The animals were restrained in right lateral recumbency; on an examination table and all the base values were recorded. Dexmedetomidine, midazolam and fentanyl in predetermined doses were injected intramuscularly into the anterior thigh muscles (biceps femoris). The animal was then left loose in a room to allow onset of the effects of the drug and to record weak time and down time. Ten minutes later the animal was secured again on examination table in right lateral recumbency and ketamine was administered in the cephalic vein until onset of anaesthesia which was confirmed by loss of pedal reflex. The study was conducted in the Division of Surgery and Referral Veterinary Polyclinic, Indian Veterinary Research Institute, Izatnagar, Bareilly, India from October 2010 to March 2011.

Table 1: System of recording of various reflexes and responses (Adapted and modified after Amarpal *et al.*, 1996)

	Parameter score				
	0	1	2	3	4
Relaxation of jaw	Not allowing to open the jaws	Resistant to opening the jaws and closed quickly	Less resistance to opening the jaws and closed slowly	No resistance and jaws remain open	-
Palpebral reflex	Intact and strong (quick blink)	Intact but weak (slow response)	Very weak (very slow and occasional response)	Abolished (no response)	-
Pedal reflex	Intact and strong (strong withdrawal)	Intact but weak (animal responding slowly)	Intact but very light (slow and occasional response)	Abolished completely (no response)	-
Response to intubation	Not permitting entry of tube in the mouth	Allowing entry but chewing	Allowing deeper entry but coughing	Difficult intubation with coughing	Easy intubation without coughing

Observation

Clinical observations: Relaxation of the jaw was taken as a measure of muscle relaxation during the study. It was evaluated by observing the resistance to opening of the jaws while pulling the jaws apart. The status of jaw relaxation was recorded at 0, 10, 15, 20, 30, 45, 60, 75, 90, 105 and 120 min intervals on a 0 to 4 score scale as shown in the Table 1. At each interval mean value for jaw relaxation score was calculated and the muscle relaxation was graded as nil on a mean score of 0, very mild when the score was >0 but <1 , mild when the score was ≥ 1 but <2 , moderate when the score was ≥ 2 but <3 and excellent when the score was 3.

Status of palpebral reflex was recorded as a measure of depth of sedation (Leppanen *et al.*, 2006). It was assessed by observing a blink of the eye lids on touching the area around medial canthus of the eyes with index finger. The status of palpebral reflex was recorded at the same intervals as for the jaw relaxation on 0 to 3 score scale as shown in the Table 1. At each interval mean value for the palpebral reflex score was calculated and the sedation was graded as absent on a mean score of 0, mild when the score was >0 but <1 , moderate when the score was ≥ 1 but <2 , deep when the score was ≥ 2 but <3 and very deep when the score was 3.

Status of pedal reflex was recorded as a measure of depth of analgesia. It was assessed by observing the withdrawal reflex to the pinching of interdigital skin of a hind foot of the animal (Kuusela, 2004). The response of the animal was graded on a 0 to 3 score scale (Table 1) at the same interval as for the jaw relaxation. At each interval mean value for pedal reflex score was calculated and the analgesia was graded as no analgesia on a mean score of 0, very mild analgesia when the score was >0 but <1 , mild analgesia when the score was ≥ 1 but <2 , moderate analgesia when the score was ≥ 2 but <3 and complete analgesia when the score was 3.

Response to intubation was recorded to assess the status of laryngeal reflexes and feasibility of intubation during different stages of sedation/anaesthesia in all the animals. The response to intubation was recorded by attempting intubation at the same intervals as for the jaw relaxation. The response of the animals to intubation was recorded at the same intervals as for the jaw relaxation using a 0 to 4 score scale as given in Table 1. At each interval mean value for intubation score was calculated and the status of laryngeal reflex was graded as strong on a score of 0 to <1 , very mild depression when the score was ≥ 1 but <2 , mild depression when the score was ≥ 2 but <3 , moderate depression when the score was ≥ 3 but <4 and complete depression of laryngeal reflex when the score was 4.

Weak time was recorded as the time elapsed from the time of injection of the drugs to the time of onset of incoordination/ataxia or drowsiness. Down time was recorded as the time that elapsed

between the time of injection of the drugs and the time when the animal attained sternal recumbency. The time to the return of righting reflex was recorded as the time elapsed from the injection of drug until the animal was able to regain sternal recumbency. Standing recovery time was recorded as the time elapsed from the time of injection of the drugs until the animal attained standing position. Complete recovery was recorded as the time elapsed from injection of the drug (s) to the time when the animal stood and walked unassisted.

Other observation like urination, vomition and defaecation, if any, were also recorded.

Physiological observations: Heart Rate (HR), Respiratory Rate (RR), Rectal Temperature (RT) and SpO₂ were recorded before administration of the drug (s) at 0 min and at 10, 15, 20, 30, 45, 60, 75, 90, 105 and 120 min after administration of the drugs. Heart rate was recorded from pulse oximeter. Respiratory rate was measured by counting and recording the excursion of thoraco-abdomen. Rectal temperature was recorded with the help of a digital thermometer, the tip of which was placed deep into the rectum as per the standard procedure. Oxygen Saturation (SpO₂) was measured by means of a pulse oximeter (Model 8600, pulse oximeter; Nonin Medical Inc. MPLS, MN). The probe of the instrument was applied to the toe web of the forelimb of the animal after clipping the hair around the site and cleaning with 70% alcohol (Huss *et al.*, 1995). Mean arterial blood pressure was recorded by placing the cuff of the Non Invasive Blood Pressure (NIBP) monitor (Surgivet[®], Smith's medical PM, Inc. Waukesha, USA) around the hind limb; with the centre of the cuff's bladder over cranial tibial artery.

Statistical analysis: The data were analyzed for statistical significance using SPSS software version 15.0 (SPSS, Inc., Chicago, IL). One way analysis of variance and Duncan's Multiple Range Test (DMRT) were used to compare the means at different time intervals among different groups. Paired "t" test was used to compare the mean values at different intervals with their base values in each group. The subjective data generated from the scoring of various parameters were analyzed using Kruskal Wallis test. In each analysis, the differences were considered significant at a value of $p < 0.05$.

RESULTS

Excellent muscle relaxation was observed at 10 min interval in all the animals which persisted up to 30 min in groups A and B and up to 75 min in group C. At 120 min interval muscle relaxation was moderate in group B and nil to very mild in groups A and C.

The palpebral reflex was almost abolished and score was suggestive of excellent sedation from 10 to 60 min followed by only mild sedation attained 0 at 120 min in group A. Almost similar scores were recorded in the animals of groups B and C, suggesting excellent sedation. Comparison among the groups did not reveal any statistically significant difference in mean palpebral reflex score at most of the time interval but at 120 min time interval. The palpebral reflex score of group A was significantly lesser from that of groups B and C.

In the animals of all the groups, the pedal reflex was lost completely after the administration of drugs at 10 min (score 3) but pedal reflex score returned at 45 min in groups A and B and 60 min in group C. Thereafter reflex score decreased gradually up to end of the study (Fig. 1).

In animals of group A, only very mild to mild depression of laryngeal reflex was observed and only 25% of the animals permitted intubation. In animals of group B, laryngeal reflexes were lost completely and all the animals permitted intubation with in 15 min interval which persisted up to

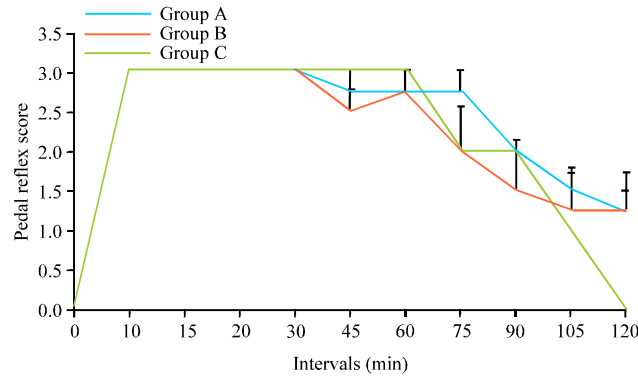


Fig. 1: Pedal reflex score in the animals of groups A (dexmedetomidine 10 $\mu\text{g kg}^{-1}$ -midazolam-ketamine), B (dexmedetomidine 20 $\mu\text{g kg}^{-1}$ -midazolam-ketamine) and C (dexmedetomidine 10 $\mu\text{g kg}^{-1}$ -midazolam-ketamine) at different time intervals

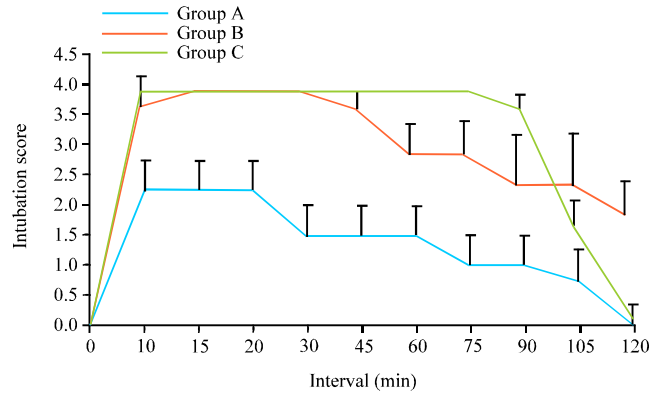


Fig. 2: Intubation score in the animals of groups A (dexmedetomidine 10 $\mu\text{g kg}^{-1}$ -midazolam-ketamine), B (dexmedetomidine 20 $\mu\text{g kg}^{-1}$ -midazolam-ketamine) and C (dexmedetomidine 10 $\mu\text{g kg}^{-1}$ -midazolam-ketamine) at different time intervals

45 min interval. In animals of group C, laryngeal reflexes were lost rapidly and all the animals permitted intubation with in 10 min interval which persisted up to 75 min interval (Fig. 2). Comparison among groups revealed significantly higher intubation scores at 10 and 90 min intervals in groups B and C than groups A. At 120 min interval intubation scores of groups B were significantly greater than that of groups A and C.

Induction dose of ketamine did not differ significantly between the groups, however, higher anaesthetic dose was required in group C ($9.76 \pm 0.64 \text{ mg kg}^{-1}$) as compared to group A ($9.04 \pm 0.059 \text{ mg kg}^{-1}$) and group B ($8.93 \pm 0.83 \text{ mg kg}^{-1}$).

Shortest weak time was recorded in the animals of group B ($3.25 \pm 0.25 \text{ min}$) followed, in increasing order, by, group C ($3.75 \pm 0.25 \text{ min}$) and group A ($4.37 \pm 0.23 \text{ min}$). Mean weak time of group A was significantly greater ($p < 0.05$) than that of group B but did not differ significantly ($p > 0.05$) from group C. Similarly, shortest down time was recorded in the animals of group B ($4.50 \pm 0.28 \text{ min}$) followed, in increasing order, by group C ($5.75 \pm 0.25 \text{ min}$) and group A ($5.87 \pm 0.125 \text{ min}$). Down time in the animals of group B was significantly ($p < 0.05$) shorter than that in groups A and C. Shortest recovery time was recorded in the animals of group A ($51.75 \pm 4.49 \text{ min}$)

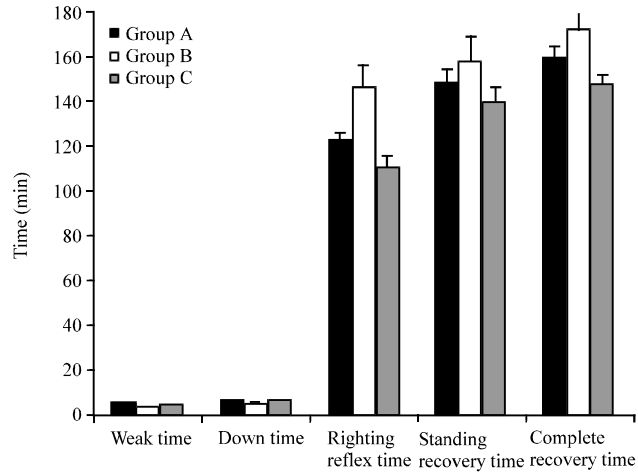


Fig. 3: Weak time, down time, righting reflex time, standing recovery time and complete recovery time in the animals of group A (dexmedetomidine 10 $\mu\text{g kg}^{-1}$ -midazolam-ketamine), B (dexmedetomidine 20 $\mu\text{g kg}^{-1}$ -midazolam-ketamine) and C (dexmedetomidine 10 $\mu\text{g kg}^{-1}$ -midazolam-ketamine) at different time intervals

followed by that in group B (74.50 ± 5.42 min) and group C (74.75 ± 0.25 min). Recovery time in the animals of group A was significantly ($p < 0.05$) shorter than that in groups B and C. Mean sternal recumbency time in group A was 123.25 ± 2.28 min, in group B 146.50 ± 10.33 min and in group C 105.0 ± 4.73 min. The sternal recumbency time in group B was significantly ($p < 0.05$) longer than that in group A and C. Shortest standing recovery time was recorded in the animals of group C (140.50 ± 6.13 min) followed, in increasing order, by that in group A (148.50 ± 5.51 min) and in group B (158.75 ± 10.71 min). Standing recovery time in the animals of group C was significantly ($p < 0.05$) shorter than that in groups A and B (Fig. 3). The complete recovery time of group C was significantly ($p < 0.05$) lower when compared to other groups. The duration of anaesthesia was longer in group C (60 min) than groups A and B (45 min).

PHYSIOLOGICAL OBSERVATIONS

In animals of group A, heart rate decreased non-significantly ($p > 0.05$) throughout the study period but a significant ($p < 0.01$) decrease in heart rate was recorded in group B at 10 min after the drugs administration. Heart rate showed some improvement at 30 min, but remained significantly ($p < 0.01$) lower than the base value up to 120 min.

In animals of group C, heart rate decreased significantly ($p < 0.01$) at 10 min after the administration of the drugs and continued to stay significantly ($p < 0.01$) below the baseline up to 120 min except at 15 and 45 min where changes in HR were non-significant ($p > 0.05$). Results indicated maximal depression of HR in group C followed by groups A and B (Table 2).

In animals of groups A and B, a significant ($p < 0.05$) decrease in RR was recorded throughout the study after the drug administration as compared to baseline. In the animals of group C, respiratory rate showed only slight and non-significant decrease throughout the study period except at 30 min interval, where the mean value was significantly ($p < 0.05$) below the baseline. Results indicated maximal depression of RR was in the animals group B and minimal in the animals of group C (Table 3).

Table 2: Mean±SE of HR in the animals of groups A (dexmedetomidine 10 µg kg⁻¹ -midazolam-ketamine), B (dexmedetomidine 20 µg kg⁻¹ -midazolam-ketamine) and C (dexmedetomidine 10 µg kg⁻¹ -midazolam-ketamine) at different time intervals

Time intervals (min)						
Groups	0	10	15	20	30	45
A	101.00±12.65	66.50±14.97	107.00±22.18	83.50±9.84	86.50±7.29	75.25±10.29
B	105.50±8.14	48.75±9.10	93.00±13.41	88.50±11.90	77.00±8.82	56.75±9.10*
C	126.00±5.15	79.50±9.85**	111.75±2.25	68.50±2.72**	63.00±12.50**	61.50±3.27

Time intervals (min)					
Groups	60	75	90	105	120
A	73.25±8.66 ^a	65.20±8.13	68.25±7.89	69.50±6.03 ^a	76.50±3.00 ^a
B	43.75±1.37 ^{b**}	41.25±1.75 [*]	44.25±4.55 [*]	40.00±1.15 ^{b**}	41.75±2.50 ^{b**}
C	50.50±3.06 ^{ab**}	44.00±4.72 ^{**}	45.75±7.76 ^{**}	54.25±10.14 ^{ab**}	56.00±15.51 ^{b**}

*Significantly different from base value (p<0.05), **Significantly different from base value (p<0.01), Values with different alphabets differ significantly at respective intervals (p<0.05)

Table 3: Mean±SE of respiratory rate in the animals of groups A (dexmedetomidine 10 µg kg⁻¹ -midazolam-ketamine), B (dexmedetomidine 20 µg kg⁻¹ -midazolam-ketamine) and C (dexmedetomidine 10 µg kg⁻¹ -midazolam-ketamine) at different time intervals

Time intervals (min)						
Groups	0	10	15	20	30	45
A	33.50±7.54	16.00±2.65 [*]	14.25±0.85	14.00±1.35	15.00±1.78 [*]	14.75±2.50 [*]
B	34.50±5.04	13.25±0.75 [*]	12.25±0.63 [*]	11.50±0.96 [*]	11.25±0.85 [*]	10.50±1.20 [*]
C	28.25±4.33	17.50±2.87	14.00±2.12	12.25±1.65	10.25±2.29 [*]	11.50±1.71

Time intervals (min)					
Groups	60	75	90	105	120
A	14.75±3.00 [*]	15.00±2.65 [*]	15.00±3.37 [*]	15.50±4.25 [*]	21.50±8.75 [*]
B	10.50±1.20 [*]	10.50±1.20 [*]	11.25±0.95 [*]	10.50±0.65 [*]	11.00±1.08 [*]
C	12.25±2.06	12.00±1.87	12.50±1.85	11.75±2.14	11.75±2.14

*Significantly different from base value (p<0.05), **Significantly different from base value (p<0.01), Values with different alphabets differ significantly at respective intervals (p<0.05)

RT decreased significantly (p<0.05) from 75 min until 120 min in group A. In the animals of group B, RT increased significantly (p<0.01) at 10 and 15 min time interval and then it decreased and remained just below the base line throughout the observation period. Rectal temperature did not vary significantly from the base value at various time intervals in the animals of group C.

Oxygen saturation values did not differ significantly (p>0.05) throughout the study period in all the groups as compared to respective baseline values except at 30 and 45 min interval in group B and 105 min interval in group C. Significantly (p<0.05) lower SpO₂ values were observed in group C at 10 and 45 min interval as compared to groups A and B. No significant (p>0.05) differences in the oxygen saturation were found among the groups at any other corresponding time intervals.

MAP increased initially and then decreased towards the end of study in all the groups. In animals of group A, mean arterial pressure varied non-significantly (p>0.05) following the drug administration up to 45 min of observation period. The MAP decreased significantly (p<0.05) below the baseline at 60 min followed by further significant (p<0.01) decrease at 90 min interval. Thereafter MAP improved until the end of observation period. Mean arterial

Table 4: Mean±SE of mean arterial pressure (mm Hg) in the animals of groups A (dexmedetomidine 10 µg kg⁻¹ -midazolam-ketamine), B (dexmedetomidine 20 µg kg⁻¹ -midazolam-ketamine) and C (dexmedetomidine 10 µg kg⁻¹ -midazolam-ketamine) at different time intervals

Time intervals (min)						
Groups	0	10	15	20	30	45
A	116.75±6.70 ^{ab}	118.25±8.22 ^a	117.75±11.56 ^a	119.80±12.37	120.50±10.31	122.00±10.54
B	128.25±8.37 ^b	113.07±15.25 ^b	157.00±9.84 ^b	160.00±14.42 [*]	131.00±4.69	131.50±8.79
C	102.25±3.17 ^a	122.25±4.97 ^a	124.00±8.05 ^a	122.50±4.66	118.50±1.84	120.05±2.53 [*]
Time intervals (min)						
Groups	60	75	90	105	120	
A	106.25±8.73 [*]	111.25±10.88	105.00±5.87 ^{**}	107.25±8.13	110.50±9.04 ^a	
B	114.42±4.09	111.50±1.65	121.50±6.53	118.25±9.49	120.50±5.17 ^a	
C	107.25±3.47	108.00±4.49	109.50±7.19	131.50±14.52	147.25±6.40 ^{b*}	

*Significantly different from base value (p<0.05), **Significantly different from base value (p<0.01), Values with different alphabets differ significantly at respective intervals (P<0.05)

pressure varied around the baseline values throughout the study period in the animals of group B, except at 20 min, where a significant (p<0.05) increase was observed. Similar to group B, MAP in the group C animals fluctuated around the baseline values except at 45 and 120 min intervals, where a significant (p<0.05) increase in MAP was observed (Table 4).

DISCUSSION

Jaw relaxation signifies the extent of muscle relaxation. In animals of all the groups good muscle relaxation was observed. All alpha-2 agonists, including dexmedetomidine are known to produce good muscle relaxation (Lemke, 2004) which is attributed to inhibition of intraneuronal transmission of impulses by alpha-2 agonists at the level of CNS (Marjorie, 2001). A good jaw relaxation has been reported after administration of dexmedetomidine in human patients (Hanci *et al.*, 2010). Midazolam, a benzodiazepine derivative is known to have good muscle relaxant action (Hellyer *et al.*, 1991; Ilkew *et al.*, 1998). Increasing the dose of dexmedetomidine up to 20 µg did not have appreciable effect on the quality of muscle relaxation during the peak effect. Greater muscle relaxation was achieved in group C as compared to groups A and B. Although, opioids by themselves do not induce muscle relaxation, however, synergistic interaction with benzodiazepine and/or alpha-2 agonist might have caused enhanced muscle relaxation in group C as compared to groups A and B. The findings of the present study conformed to the observations of earlier researchers who reported greater muscle relaxation when dexmedetomidine or medetomidine was combined with opioid and/ or ketamine in cats or dogs (Ko *et al.*, 2000; Selmi *et al.*, 2003).

Status of palpebral reflex was taken a measure of sedation. Excellent sedation was recorded in all the groups and no significant differences were found between the groups during the peak effects, however, palpebral reflex score was higher in groups B compared to group A towards the end of observation period. In the earlier studies also, no significant differences were reported in the sedative scores of dogs given dexmedetomidine-diazepam, dexmedetomidine-butorphanol or dexmedetomidine-buprenorphine (Leppanen *et al.*, 2006) or medetomidine alone, medetomidine-butorphanol or medetomidine-ketamine were compared (Ko *et al.*, 2000). Almost similar scores in groups A and B during most of the period conformed to the earlier observations that increasing the

dose of dexmedetomidine beyond certain level does not cause a further increase in sedation (Kuusela *et al.*, 2000).

Some part of the sedation in all the groups might be attributed to the action of midazolam. Although midazolam is a mild sedative agent for dogs, yet it shows synergistic activity when administered with other sedatives as reported by Cwiek *et al.* (2009). Synergistic interaction has been recorded between midazolam and dexmedetomidine in earlier studies also (Bol *et al.*, 2000). Leppanen *et al.* (2006) has reported some limitations of palpebral reflex in assessment of sedation in dogs under general anaesthesia. But in the present study evaluation of palpebral reflex gave a fair idea of the depth of the sedation and it was possible to differentiate between mild, moderate and deep sedation.

The depth of the analgesia did not differ between groups A and B. Administration of ketamine produced complete abolition of pedal reflex in both the groups at 10 min which lasted up to 30 min interval. Hayashi *et al.* (1995) reported that analgesia produced by dexmedetomidine is mediated at the spinal level where it interrupts nociceptive pathways to the ventral root of dorsal horn which reduces spinal reflexes (Kending *et al.*, 1991; Savola *et al.*, 1991). Midazolam is also reported to have a considerable effect on the nociceptive transmission in superficial dorsal horn (Kohno *et al.*, 2006). Intramuscular midazolam has been reported to cause pain relief especially in postoperative period (Akhlaghi and Rajaei, 2008). The analgesia achieved in groups C was deeper and of longer duration than that of groups A and B which could be attributed to the action of μ opioid agonist, fentanyl which is about 80 to 100 times more potent than morphine. Thurmon *et al.* (1999) also to synergistic interaction between alpha-2 agonists, opioids and benzodiazepines (Salmenpera *et al.*, 1994; Amarpal *et al.*, 1996; Bol *et al.*, 2000). Fentanyl provided extended period of analgesia than ketamine when used for postoperative analgesia in children undergoing adenotonsillectomy (Taheri *et al.*, 2011). The combination of pethidine, an opioid, with ketamine has been shown to effectively reduce postoperative pain (Nourozi *et al.*, 2010). The complete sedation and analgesia in the animals of all the groups during peak effect could be attributed to the general anaesthesia induced by ketamine after premedication of dexmedetomidine-midazolam in groups A and B and dexmedetomidine-midazolam-fentanyl in group C.

Dexmedetomidine and midazolam, by themselves are not general anaesthetics and incapable of completely abolishing the laryngeal reflex and also laryngeal and pharyngeal reflexes are reasonably well maintained during ketamine induced anaesthesia in all species (McCarthy *et al.*, 1965; Haskins *et al.*, 1975). This might have prevented intubation in group A. Increase in the dose of dexmedetomidine along with reported synergism between midazolam and dexmedetomidine (Bol *et al.*, 2000) might have supervened the effects of ketamine to allow intubation in the animals of group B. In group C, a complete depression of laryngeal reflex might have been achieved due to synergistic interaction of dexmedetomidine and midazolam with fentanyl (Ben-Shlomo *et al.*, 1990; Salmenpera *et al.*, 1994).

Weak time represents the time of onset of action of sedative drugs. In the animals of group A, the weak time was almost similar to that reported earlier for medetomidine or dexmedetomidine in dogs (Amarpal *et al.*, 1996; Ahmad *et al.*, 2011). The rapid onset of effects of medetomidine has been attributed to its lipophilic property (Amarpal *et al.*, 1996; Singh *et al.*, 2005). Higher dose of dexmedetomidine in group B as compared to group A could be responsible for shorter weak time in group B compared to group A. The synergistic interaction of midazolam and fentanyl with dexmedetomidine might have contributed to the decrease in the weak time in the animals of group C as compared to that in groups A and B. When drugs acting on different sites are administered simultaneously, a synergistic action may be expected. Amarpal *et al.* (1996) reported a reduced weak time when pentazocine was administered with medetomidine as compared to medetomidine

alone. Due to rapid onset of effects of dexmedetomidine a short down time was recorded (5.87 ± 0.125 min) in the animals of group A which is similar to that of medetomidine as reported by Pratap *et al.* (1997). Further, because of simultaneous actions of dexmedetomidine, midazolam and fentanyl, decrease in down time was recorded in the animals of groups B and C which might have acted at different sites in the CNS leading to faster onset of CNS depression.

Anaesthesia was induced after administration of ketamine in all the groups and induction dose of ketamine did not differ between the groups. Ketamine is a short acting anaesthetic drug (Stephenson *et al.*, 1978). The recovery from ketamine is reported to occur through tissue redistribution and hepatic metabolism (Kaka and Hayton, 1980). The prolonged recovery time recorded in all the groups might be due to the synergistic actions of dexmedetomidine, midazolam and fentanyl. Significantly longer recovery time in groups B and C as compared to group A could be due to higher dose of dexmedetomidine in group B and addition of fentanyl in group (Kuusela *et al.*, 2000) reported that recovery time was prolonged when dogs were treated with IV medetomidine $40 \mu\text{g kg}^{-1}$ and dexmedetomidine $20 \mu\text{g kg}^{-1}$, as compared with the lower dose levels. Similarly, in the present study recovery time were longer in the animals of group B as compared to groups A and C.

As reported by Cwiek *et al.* (2009), dogs premedicated with midazolam-xylazine needed a shorter time to regain consciousness and motor functions compared to those premedicated with xylazine alone. A significantly longer sternal recumbency time was observed in cats given dexmedetomidine with ketamine, than dexmedetomidine alone or dexmedetomidine-butorphanol group (Selmi *et al.*, 2003). The results conformed to the observations of Ueyema *et al.* (2008), who recorded 20 to 60 min duration of anaesthesia and analgesia in dogs administered with medetomidine-morphine-ketamine combination intramuscularly.

Alpha-2 agonist associated decrease in the HR is attributed mainly to reflex bradycardia due to vasoconstriction (Lemke, 2004). Dexmedetomidine causes profound bradycardia but preserves blood pressure when administered intravenously (Kuusela *et al.*, 2001). Greater depression in the heart rate in the group B and C as compared to group A might be attributable to higher dose of dexmedetomidine in group B and addition of fentanyl in group C. Dose dependent cardiovascular effects of medetomidine/dexmedetomidine have been described earlier (Pypendop and Versteegen 1998). Bradycardia following fentanyl administration is associated with strong activation of cardiac vagal efferents (Thurmon *et al.*, 1999).

Administration of medetomidine or dexmedetomidine has been found to decrease respiratory rate with minimal effects on blood gases in dogs (Amarpal *et al.*, 1996; Kuusela *et al.*, 2000). Oyamada *et al.* (1998) opined that inhibition of locus coeruleus neurons by activation of alpha-2 adrenergic pathway might be responsible for alpha-2 agonist induced RR depression. Similarly midazolam, fentanyl and ketamine are known to depress respiratory rate. Thus greater depression in RR in groups B and C as compared to group A is attributed to higher dose of dexmedetomidine and action of fentanyl, respectively.

Decrease in rectal temperature after the onset of the effects of sedative/anaesthetic drugs might be attributed to decrease in heat production due to decreased muscular activity and direct effect of the drugs on hypothalamus (Virtanen, 1989). A gradual decrease in rectal temperature following intravenous administration of dexmedetomidine was observed by Raekallio *et al.* (2005). Also as observed by Wright (1982), decrease in RT might be attributed to a direct depression of thermoregulatory centre of hypothalamus by ketamine.

In spite of decrease in RR, SpO₂ was fairly maintained in all the groups. Occasional low pulse oximeter readings could be due to technical error (Leppanen *et al.*, 2006).

Dexmedetomidine has a contrasting impact on blood pressure, because it nonselectively stimulates alpha-2A and alpha-2B adrenergic receptors (Ebert *et al.*, 2000). In all the groups in the present study, an initial increase in MAP was followed by a decrease in MAP. Initial increase in MAP could be attributable to peripheral action of dexmedetomidine. As the drug is metabolized, a decrease in blood pressure could be due to predominantly central action of dexmedetomidine (Sano *et al.*, 2010). Administration of midazolam in dogs is known to cause a significant decrease in arterial pressure (Butola and Singh, 2007) but fentanyl itself does not cause a significant decrease in the mean arterial blood pressure (Grimm *et al.*, 2005). The decrease in blood pressure conformed to the observations of Sano *et al.* (2010) who reported consistent decrease in arterial blood pressure when dexmedetomidine was used with other preanaesthetics/anaesthetics in swine. However, the maintenance of MAP within physiological limits in most of the groups was attributed mainly to the administration of ketamine as it produces an increase in cardiac output and heart rate with a significant increase in B.P. (Zielmann *et al.*, 1997).

It was concluded that dexmedetomidine (10 µg kg⁻¹)-midazolam (0.4 mg kg⁻¹) ketamine combination produced excellent muscle relaxation and anaesthesia for 45 min with only minor changes in cardiorespiratory parameters. Increasing the dose of dexmedetomidine to 20 µg kg⁻¹ did not offer any advantage except for slight faster onset and prolonged post anaesthetic sedation. Addition of fentanyl (4 µg kg⁻¹) not only increased the duration of anaesthesia with minimal changes in cardiorespiratory parameters but also facilitated the recovery.

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