COMPARATIVE EVALUATION OF THE EPIDURAL DEXMEDETOMIDINE, KETAMINE OR FENTANYL IN COMBINATION WITH BUPIVACAINE IN DOGS

[1]Eman M. Nour, [2]Mahmoud Mahmoud Othman,
[3]Gamal I.A. Karrouf and [4]Adel E.I. Zaghloul

[1]Animal Research Facility, Urology and Nephrology Center,
Faculty of Medicine, Mansoura University, Mansoura, Egypt
[2]Department of Anesthesia and Surgical ICU,
Faculty of Medicine, Mansoura University, Mansoura, Egypt
[3,4]King Fahd Medical Research Center,
King Abdulaziz University, P.O. Box: 80216, Jeddah 21589, Saudi Arabia
[4]Department of Surgery, Anesthesiology and Radiology,
Faculty of Veterinary Medicine, Mansoura University, Mansoura, Egypt

ABSTRACT

Castration is one of the most common procedure. It is a potential source of mild to moderate pain which is enough to induce inflammatory process, necessitate the incorporation of analgesics. This study was designed to evaluate the effect of epidural bupivacaine alone or in association with fentanyl, ketamine or dexmedetomidine on analgesia for the first 24 h post surgical castration. Forty male mongrel dogs were randomly allocated into 4 groups received bupivacaine 0.25% (1.5 mg kg^{-1}) alone or in combination with fentanyl citrate (2 µg kg^{-1}) or ketamine hydrochloride (2 mg kg^{-1}) or dexmedetomidine hydrochloride (1.13 µg kg^{-1}) into the lumbosacral space. Dogs were scored for sedation using numeric descriptive scale. Analgesia was assessed subjectively through Modified University of Melbourne Pain Scale score and objectively by following up the circulating level of interleukins 6 and 10. The bupivacaine-dexmedetomidine group showed the greatest sedation score, the lowest pain score without neuroendocrine modulation in contrast with bupivacaine or bupivacaine-fentanyl groups. From these result, it is concluded that bupivacaine-dexmedtomidine group provide long term analgesia last for the first 24 h post operative without additional analgesics.

Keywords: Bupivacaine, Ketamine, Dexmedetomidine, Intereukins, Fentanyl

1. INTRODUCTION

Castration of male dogs is frequently performed in veterinary practice. It is assumed to be a mild to moderate painful procedure (Mathews, 2000). It induces inflammatory process followed by neuroendocrine modulation such as increase in serum levels of acute phase proteins and decrease in serum levels of insulin like growth factor I and adiponectin (Tvarijonaviciute *et al.*, 2011).

Post operative pain has deleterious effects that hinder the recovery of the patient. Thus post operative analgesia is of utmost importance (Hansen, 2005) and should start as early as possible. Post operative pain is presumed to peak during the first 24 h after surgery and this is the time period studied in most clinical trials (Slingsby *et al.*, 2006).

Epidural anesthesia is considered an extremely safe procedure. It can be used efficiently for a wide variety of

Corresponding Author: Eman M. Nour, Animal Research Facility, Urology and Nephrology Center, Faculty of Medicine,
Mansoura University, Mansoura, Egypt

surgical procedure to provide intra and post operative analgesia. Epidural anesthesia is preferable than general anesthesia; lower mortality and morbidity, no need for orotracheal intubation, better post operative analgesia and a decrease in surgical stress through blockade of afferent nociceptive pathways and efferent sympathetic impulses.

Based on previous literatures stating that the provided analgesia after epidural lidocaine/tramadol extended up to the 12 h post castration (Almeida *et al*., 2010) or as (Slingsby *et al*., 2006) stressing the need to re-administration of a second dose buprenorphine at either four or 6 h after the first enhanced level of analgesia. Both clarified the short term analgesic effect of different analgesics.

This study is designed to evaluate the usefulness of different epidural regimens to provide prolonged post operative analgesia extended up to the first 24 h post castration. These compared regimens based on local anesthetic bupivacaine in combination with; Fentanyl, ketamine or dexmedetomidine. Assuming that epidural dexmedetomidine-bupivacaine analgesia will be superior to other combinations analgesia.

2. MATERIALS AND METHODS

2.1. Animals

Forty male mongrel dogs were kept under controlled environment in the Animal Research Facility Unit, Urology and Nephrology Centre, Mansoura University. Physical examination was used to determine whether the dogs met the inclusion criteria of normal results of neurologic examination, normal biochemical profile normal skin over the epidural injection site and absence of known systemic diseases. None had received previous analgesic treatment. The study was approved by local ethical committee of the faculty of Veterinary Medicine, Mansoura University.

2.2. Study Design

The dogs were randomly allocated into 4 groups of 10 each in advance of the study and in order to keep the blindness of the experiment one of colleagues had the key of randomization and was responsible for preparing the different epidural combination served in a similar syringe.

All the assigned groups received bupivacaine 0.25% 1.5 mg kg^{-1} (0.6 mL kg^{-1}) (Bucaine 0.5%, vial Weimar, German) (Freire *et al*., 2010) in combination with adjuvants according to randomization; Bupivacaine Group (BG): 1 mL placebo; Ketamine Group (BKG): Ketamine HCL 2 mg kg^{-1} (Hamilton *et al*., 2005); Fentanyl Group (BFG): Fentanyl HCL 2 µg kg^{-1} (Almeida *et al*., 2007);

Dexmedetomidine Group (BDG): Dexmedetomidine HCL 1.13 µg kg^{-1} (Sabbe *et al*., 1994).

2.3. Surgical Procedure

The dog was muzzled to preventbiting, placed on a stainless steel table; clipping and antiseptic preparations were performed on the lumbar region before administration of the epidural agents. A 20 gauge needle was inserted into the lumbosacral space by the technique described by Jones (2001) the correct positioning of the needle was confirmed by the absence of cerebrospinal fluid or blood at the hub and by the hanging drop technique.

Dogs were maintained in sternal recumbency for at least 15 min to facilitate the uniform distribution of the drugs. Immediately before the beginning of the surgical procedure. A catheter was inserted into the cephalic vein for administration of propofol 7 mg kg^{-1} IV, followed by continuous infusion of the same agent 0.2 mg kg^{-1} min that was adjusted as necessary to maintain a light plane of anesthesia, Lactated Ringer's solution at a rate of 10 mL kg^{-1} h was administered during the surgical procedure (Almeida *et al*., 2007). Then dogs were positioned in dorsal recumbency.

The motor blockade was indicated by motor incoordination of the limb till complete paralysis. Sensory blockade was indicated by absence of groaning, biting attempts, looking at the limb and head shaking by painful stimulus (pressure on interdigital space of hind foot performed with a hemostats protected with rubber) after administration of epidural anesthetics and every 2 min.

All castrations were performed by the same surgeon performed 30 min after the epidural injection. The incision is made in the skin in front of the scrotum. Each testicle is pushed up through this incision, spermatic cord were clamped then legated with silk (2/0) and transected off. The subcutaneous tissue is sutured using the polygalactin 910 (vicryle Ethicon) absorbable suture materials.

2.4. Post-Operative Evaluation

Analgesia and sedation were measured postoperatively at 1, 2, 3 and 4 h post castration while analgesia continued for the first 24 h post castration.

The degrees of sedation and analgesia were evaluated by the use of Numeric Descriptive Scale (NDS) in which 0 corresponds to no sedation, (1) Mild sedation (Less alert but still active, (2) Moderate sedation (drowsy, recumbent but can walk) and (3) corresponds dogs to intense sedation (Monteiro *et al*., 2009) and the scale

proposed by Modified University of Melbourne Pain Scale (UMPS) (Holton *et al*., 1998) in which 0 corresponds to no pain and 23 to the worst pain possible. Dogs considered being in pain if UMPS equal 8 or more. Dogs were evaluated by the same observer who was unaware of group assignments, which permitted unbiased evaluation of the analgesic effects of the drug.

2.5. Serum Interleukin-6 (IL-6) and Interleukin-10 (IL-10)

Blood samples were collected at T_0, T_{4h} and T_{24h} post operative. Immulite 1000 Analyzer was used for the quantitative measurement of IL-6 and IL-10 in serum. For IL-6 kits the absolute range was nondeductible to 5.9 pg mL^{-1} (590 ng dL^{-1}) from simens and for IL-10 immulite kits median reference range was 1.5 pg mL (150 ng dL^{-1}) and a 95th percentile of 9.1 pg mL^{-1} (910 ng dL) from simens.

2.6. Statistical Analysis

The data was done by using SPSS version 17.0 the data were analyzed for normality using Kolmogrov-Smirnov test. Data were represented as the mean±SD for parametric data; latency and duration of motor and sensory blockades, heart rate, non invasive blood pressure, respiratory rate, interleukin-6 and interleukin-10. While non-parametric data were represented as median (IQR) like NDS and Modified UMPS. For parametric data, comparisons were carried out by ANOVA with the least significance post hoc analysis for inter group comparison while for non-parametric data, comparisons were carried out by Kruskal-Wallis H-test followed by Wilcoxon singed rank test for multiple comparison test. Repeated measures ANOVAs were used when data were collected in multiple trials of a single session, followed by the Fisher LSD test. Significance was considered when P value <0.05.

3. RESULTS

Forty male mongrel dogs were randomly assigned into four groups: (N = 10) Bupivacaine Group (BG), Bupivacaine/Ketamine Group (BKG), Bupivacaine/Fentanyl Group (BFG) and Bupivacaine/Dexmedetomidine Group (BDG).

Epidural injection of the drugs at the lumbosacral space was easily accomplished in all the dogs and no untoward reactions/complications associated with injection were observed throughout the study.

After administration of propofol all dogs didn't show any gross movements of the forelimbs and head. No differences in the dose of propofol for each group; an induction dose of propofol (7mg kg^{-1}, IV, without other premedication), followed by a continuous infusion of the same agent (0.2 mg kg^{-1} min) maintaining light plane of anesthesia.

3.1. Motor and Sensory Blockades

For latency of motor blockade, it was (8.60±2.3 min, 2.4±0.7 min, 7.5±2.3 min and 5.1±1.9 min) for BG, BKG, BFG and BDG respectively (with a significantly shorter time for BKG then BDG compared with BG group). For BFG the latency of motor blockade was (7.50±2.3 min) longer than BKG and BDG but shorter than BG. Duration of motor blockade was (98±35.606 min, 177.73±49.768 min, 134.92±21.748 and 255± 49.554 min) for BG, BKG, BFG and BDG respectively with significant longer duration for BDG then BKG compared with BG (**Table 1**).

As well, latency of sensory blockade, it was (13.10±4.1 min, 4.27± 1.2 min, 10.1±2.9 min and 5.1± 1.7 min) for BG, BKG, BFG and BDG respectively with significant difference between BKG and BDG compared with BG. Duration of the sensory blockade was (82.50±34.420 min, 171.36±43.937 min, 126.67±19.929 and 242±46.975 min) for BG, BKG, BFG and BDG respectively with significantly longer duration for BDG, BKG then BFG when compared with BG, but for BKG and BFG; the BKG had a significantly longer duration when compared with BFG (**Table 1**).

3.2. Cytokines

Supernatant IL-6 concentration from whole blood stimulated with castration significantly differ among groups. BG and BFG showed significant increase 4 h (303.13±28.3 ng dL^{-1}), (297.13±27.7 ng dL^{-1}) and 24 h post operative (361.13±21.5 ng dL^{-1}), (353.13±19.7 ng dL^{-1}) compared with their basal values (258.75±27.2 ng dL^{-1}), (255.75±13.80 ng dL) respectively. BKG showed no change 4 h post operative (254.13±19.4 ng dL) and significant increase 24 h post operative (331.75±22.5 ng dL^{-1}) in comparison with the basal value (260.75±13.6 ng dL^{-1}). While BDG showed no significant changes 4 h (257.88±16.1 ng dL^{-1}) or 24 h post operative (261.88±24.12 ng dL^{-1}) when compared with control value (262.00±5.5 ng dL^{-1}).

The serum concentration of IL-6 in dogs with epidural injection of B or BFG were signicantly increased (p<0.05) 4 h post operative compared with BKG and BDG, but 24 h post operative IL-6 in dogs with epidural injection of B or BF or BK were highly signicantly (p<0.05) increased compared with BDG (**Table 2**).

Significant difference in the concentration of serum IL-10 was observed four and 24 h post castration. In B and BFG, normal concentration of serum IL-10 were (317.38±38 ng dL^{-1}), (320.38±62.02 ng dL^{-1}) respectively 4 h post surgical the result of serum IL-10 decreased sigificantly to be (240.25±30.041 ng dL), (245.7±13.4ng dL^{-1}) respectively, 24 h post surgical stimulus serum IL-10 displayed more significant decrease to be (190.25±22.23 ng dL^{-1}), (195.4±10.3 ng dL^{-1}) for both groups respectively.

For BKG, the pre-operative values was (341.63±37.627 ng dL), 4 h post castration, no significant difference of serum IL-10 was recorded (330.75±43.744 ng dL) but 24 h post castration serum IL-10 was significantly decreased (199.00±13.6 ng dL). Other pattern was observed in serum concentration of IL-10 in BDG where no significant difference between pre-operative value (321.25±38.6 ng dL) or 4 h (320.38±24.11 ng dL) or 24 h post castration (331.25±16.714 ng dL).

The recorded concentration of serum IL-10 4 h post castration in B and BF were significantly decreased (p<0.05) compared to the level of IL-10 belong to BK or BD groups. Twenty four hours post castration serum IL-10 in BDG was significantly (p<0.05) increased than B, BF and BK groups (**Table 2**).

3.3. Assessment of Sedation (NDS)

During the postoperative observation period in all groups, the NDS values did vary significantly with time. However, the BDG had the higher sedation values ranged from intense to moderate, which were significantly different in relation to B, BF and BK. In dogs received Bupivacaine with or without ketamine or fentanyl, sedation ranged from intense to no sedation, a decrease in all groups was observed with time (**Table 3**).

3.4. Assessment of Analgesia (UMPS)

For analgesia, according to Modified UMPS, an increase in pain scale scores in all groups were observed during the postoperative period except for BDG which showed significantly lower values than in the B, BK and BF groups (**Table 4**).

Table 1. Latency (Onset of sensory and motor blockades) and duration of motor and sensory blockades in mongrel dogs that received an epidural injection of Bupivacaine (B), Bupivacaine-Ketamine (BK), Bupivacaine-Fentanyl (BF) and Bupivacaine-Dexmedetomidine (BD) groups

	B-group (n = 10)	BK-group (n = 10)	BF-group (n = 10)	BD-group (n = 10)
Sensory Blockade				
Latency (min)	13.1±4.1	4.2±1.2$^\$$	10.1±2.9	5.1±1.7$^\$$
Duration (min)	82.5±34.4	171.3±43.9$^\$$	126.6±19.9$^{\$\#}$	242±46.9$^{\$\#\dagger}$
Motor Blockade				
Latency (min)	8.60±2.3	2.4±0.7$^\$$	7.5±2.3	5.1±1.9$^\$$
Duration (min)	98±35.6	177.7±49.7$^\$$	134.9±21.7	255±49.5$^\$$

$ significant when compared with Bupivacaine group (p<0.05), # significant when compared with Bupivacaine-Ketamine group (p<0.05), †significant when compared with Bupivacaine-Fentanyl group (p<0.05). Values are mean±SD

Table 2. Interleukin-6 and interleukin-10 levels (ng/dL) in mongrel dogs that received an epidural injection of Bupivacaine (B), Bupivacaine-Ketamine (BK), Bupivacaine-Fentanyl (BF) and Bupivacaine-Dexmedetomidine (BD) groups

ILs	Time	B-group (n = 10)	BK-group (n = 10)	BF-group (n = 10)	BD-group (n = 10)
IL- 6	Basal	258.7±27.2	260.7±13.6	255.7±13.8	262±5.5
	4 h post op.	303.1±28.3*	254.1±19.4$^\$$	297.1±27.7$^{*\#}$	257.8±16.1$^\$$
	24 h post op.	361.1±21.5*	331.7±22.5*	353.1±19.7*	261.8±24.1$^{\$\#\dagger}$
IL-10	Basal	317.3±66.2	341.6±37.6	320.3±62.0	321.2±38.6
	4 h post op.	240.2±30.0*	330.7±43.7$^\$$	245.7±13.4$^{*\#}$	320.3±24.1$^{\$\dagger}$
	24 h post op.	190.2±22.2*	199.0±13.6*	195.4±10.3*	331.2±16.7$^{\$\#\dagger}$

*significant when compared with basal value (p<0.05); $ significant when compared with Bupivacaine group (p<0.05), # significant when compared with Bupivacaine-Ketamine group (p<0.05), † significant when compared with Bupivacaine-Fentanyl group (p<0.05). Values are mean ± SD

Table 3. Numeric Descriptive Scale (NDS) sedation score in mongrel dogs that received an epidural injection of Bupivacaine (B), Bupivacaine-Ketamine (BK), Bupivacaine-Fentanyl (BF) and Bupivacaine-Dexmedetomidine (BD) groups. Used to assess sedation in surgical castrated dogs

NDS	B-group (n = 10)	BK-group (n = 10)	BF-group (n = 10)	BD-group (n = 10)
T1 h	3 (3.0-3.0)	3.0 (3.0-3.0)	3 (3.0-3.0)	3 (3.0-3.0)
T2 h	1.5(1.0-2.0)*	3 (3.0-3.00)$	2 (1.75- 2.0)*#	3 (3.0-3.0)$†
T3 h	0 (0.0-0.0)*	2 (1.0-2.0)*$	1 (0.75-2.0)*$#	3 (3.0-3.0)$#†
T4 h	0 (0.0-0.0)*	1.5(1.00-2.00)*$	0 (0.0-0.0)*#	2 (2-3.0)*$†
T5 h	0 (0.0-0.0)*	0 (0.0-0.0)*	0 (0.0-0.0)*	2 (1.0-3.0)*$#†

* significant when compared with basal value (p<0.05); $ significant when compared with Bupivacaine group (p<0.05), # significant when compared with Bupivacaine-Ketamine group (p<0.05), † significant when compared with Bupivacaine-Fentanyl group (p<0.05). Values are median (IQR)

Table 4. Modified UMPS analgesia score in mongrel dogs that received an epidural injection of Bupivacaine, Bupivacaine-Ketmine, Bupivacaine-Fentanyl and Bupivacaine-Dexmedetomidine groups. Used to assess pain in surgical castrated dogs

UMPS	B-group (n = 10)	BK-group (n = 10)	BF-group (n = 10)	BD-group (n = 10)
T1hr	3 (3.0-3.0)	0.0 (0.0-0.0)$	1 (1.0-1.25)$#	0 (0.0-0.0)$†
T2hrs	6 (4.7-6.25)*	1 (1.0-1.00)*$	3 (3.0-4.0)*$#	0 (0.0-0.0)*$#†
T3hrs	8 (6.25-10.25)*	1 (1.0-2.0)*$	5 (3.0-7.0)*$#	0 (0.0-0.0)$#†
T4hrs	9 (6.0-11.0)*	2 (1.0-2.25)*$	7 (6.0-8.5)*#	0 (0.0-0.0)$#†
T5hrs	10 (8.7-11.0)*	2 (1.0-3.0)*$	10 (9.0-11.0)*#	1 (1.0-1.0)*$†
T24hrs	14 (12.7-16.0)*	11 (10.0-2.2)*$	12 (11.7-3.0)*$	3 (2.0-3.0)*$#†

* significant when compared with basal value (p<0.05); $ significant when compared with Bupivacaine group (p<0.05), # significant when compared with Bupivacaine-Ketamine group (p<0.05), † significant when compared with Bupivacaine-Fentanyl group (p<0.05). Values are median (IQR)

4. DISCUSSION

Epidural block is one of the most popular, reliable and safe techniques in animals that can provide analgesia for a variety of surgical procedures. The main disadvantage of epidural block is the short duration of action after a single injection (Lloyd-Thomas, 1990). Thus, the use of combinations in epidural anesthesia is considered. In this study, the use of dexmedetomidine, ketamine and fentanyl as adjuvant to bupivacaine was evaluated.

In the present study, all dogs had adequate motor and sensory blockades. The use of bupivacaine alone gave late and short blockade. This was in line with De Segura *et al.* (2000) reported that latency of bupivacaine alone is to be of 20 to 30 min post epidural administration. This latency was explained by its moderate onset of action and 95% plasma protein binding activity (Lamont, 2002).

For the other three compared groups, the latency of blockade was earlier for ketamine-bupivacaine combination followed by dexmedetomidine-bupivacaine whereas it was late for fentanyl-bupivacaine when compared with bupivacaine alone.

Kathirvel *et al.* (2000) noticed that ketamine added to spinal bupivacaine had local anesthetic-sparing effects in humans. Ketamine possibly diffused from the epidural space into subarachnoid space and then to spinal nerve roots. Hustveit *et al.* (1995) reported that epidurally administered ketamine produced faster onset explained by ketamine's interaction with mu, delta and kappa opioid receptors.

However, these results were in contrast with, bupivacaine group, (Almeida *et al.*, 2007) who deduced in their study the late latency of both sensory and motor blockades of fentanyl-bupivacaine than bupivacaine 0.5% alone and also at odds with Gupta *et al.* (2011) who concluded no difference in latency of motor or sensory blockade between dexmedetomidine or fentanyl added to hyperparic bupivacaine 0.5%. Gandhi *et al.* (2012) concluded that bupivacaine 0.25% is faster in inducing motor and sensory blockades than dexmedetomidine-bupivacaine 0.25% group.

Regarding the duration of blockade, the combination of dexmedetomidine-bupivacaine gave the longest blockade. Followed by ketamine-bupivacaine then Fentanyl-bupivacaine in comparison to bupivacaine.

The prolongation of the motor block of dexmedetomidine-bupivacaine can be explained by the binding of α_2-adrenoceptor agonists to motor neurons in the dorsal horn (Harada *et al.*, 1995) and the synergism between local anesthetic and α_2-adrenoceptor agonist (especially for the 2A subtype, which causes it to be a much more effective sedative and analgesic agent (Calzada and de Artinano, 2001).

For fentanyl-bupivacaine combination, Al-Ghanem *et al.* (2009) inferred that epidural administration of morphine-fentanyl shorten the onset of analgesia because fentanyl is a lipophilic μ-receptor agonist opioid, fast onset and a short duration of action, when administerdepidurally rapidly absorbed into epidural fat and the systemic circulatory system, resulting in minimal contact time with spinal opioid receptors.

These results were in line with (Gupta *et al.*, 2011) concluding thatintrathecaldexmedetomidine is associated with prolonged motor and sensory block, hemodynamic stability and reduced demand for rescue analgesics in 24 h as compared to fentanyl. Almeida *et al.* (2007) who deduced in their study the late latency of both sensory and motor blockades of fentanyl-bupivacaine than bupivacaine 0.5% alone.

Associated bradycardia, hypotension and respiratory depression in dexmedetomidine-bupivacaine, fentanyl-bupivacaine or bupivacaine groups owing to α_2-adrenoreceptors agonist (Venn *et al.*, 2001), opioid element (Trescot *et al.*, 2008) or rostral spread of the anesthetics with subsequent desensitization of nerves in the sympathetic trunk which have a role in regulation of hemodynamic variables including heart rate and vascular tone, branch off from the thoracolumbar region of the spinal cord (Perez *et al.*, 2013).

NDS, the sedation score used, revealed that dexmedetomidine-bupivacaine combination had the greatest sedation (ranged between intense to moderate allover 4 h post operative) followed by ketamine-bupivacaine and fentanyl-bupivacaine cominatrions (their sedative ranged from intense to no sedation by the 4 h of observation). bupivacaine had the least sedation, as no sedation was recorded 2 h postoperative. The sedation was diminished before complete return of pain sensation in the four groups.

Probably, the high values of sedation obtained in combination groups were mostly related to the used anesthetic (propofol). Adding dexmedetomidine, an α_2-Agonist, promotes sedation by stimulating the locus coeruleus, a part of the brain stem involved in the sleep-wake cycle and inhibition of the sympathetic vasomotor center of the brain (Kemp *et al.*, 2008).

However, the sedative effect of dexmedetomidine seemed to decrease over time. Dogs receiving dexmedetomidine appeared quiet and relaxed throughout the study, but easily rousable when interaction was required. This effect has also been described in humans where the sedative effect of dexmedetomidine has been described as arousable or co-operative sedation (Gerlach and Dasta, 2007).

In the present study, dogs in the dexmedetomidine-bupivacaine group had significantly the lowest pain scores according to the subjective evaluation using the UMPS compared with dogs in other groups. Dogs in the bupivacaine group were the earliest to express signs of acute pain followed by dogs in fentanyl-bupivacaine group. Whereas Dogs in ketamine-bupivacaine showed high pain scores 24 h post operative.

These findings were in line with, Singh *et al.* (2012) estimating that clonidine (an α_2-agonist) in a dose of 1 μg kg^{-1}, added to 0.25% bupivacaine for caudal analgesia, for sub-umbilical surgery, significantly prolongs the duration of post-operative analgesia when compared to 0.25% bupivacaine in normal saline than 0.75 mL kg^{-1} of 0.25% bupivacaine with ketamine 0.5 mg kg^{-1} or 0.75 mL kg^{-1} of 0.25% bupivacaine with fentanyl 1 mcg kg^{-1} or 0.75 mL kg^{-1} of 0.25% bupivacaine alone, without any side effects. Saadawy *et al.* (2009) as they concluded that caudal dexmedetomidine seems to be a promising adjunct to provide excellent analgesia without side effects over a 24 h period.

In contrast with the results of the present study, (Cook *et al.*, 1995) comparing the effects of adrenaline, clonidine and ketamine in adjuvant with bupivacaine on the duration of caudal analgesia in children concluded that ketamine produced longer duration of analgesia compared to clonidine (an α-agonist) and adrenaline. Almeida *et al.* (2007) as their dogs administered with the same medications but with different concentration of bupivacaine (0.5%) and showed lower pain scores and rescue medication was not needed up to 360 min after epidural injection, this perhaps due to preanesthetic medication acepromazine administered by 0.1mg kg^{-1} IM.

The analgesic effect of the α_2 agonists is a complex issue, they can induce analgesia by acting at three different sites: In the brain and brain stem, spinal cord and in peripheral tissues. α_2-adrenergic agonists act on locus coeruleus, representing a supraspinal site of action. In the spinal cord, their analgesic effect is related to activation of the descending medullospinal noradrenergic pathways or to the reduction of spinal sympathetic outflow at presynaptic ganglionic sites (Arian *et al.*, 2004).

Ketamine has analgesic properties that are mediated by a number of mechanisms. NMDA receptor noncompetitive antagonism accounts for most of its analgesic effects through a use-dependent channel blockade (Visser and Schug, 2006). The affinity of ketamine for NMDA receptors is several-fold higher than that for μ receptors, non-NMDA glutamate receptors,

nicotinic and muscarinic cholinergic receptors and monoaminergic transporter sites (McCartehy *et al.*, 2004).

In the present study, dogs in the dexmedetomidine-bupivacaine group showed no neuroendocrine modulation for the first 24 h post operative according to the objective evaluation using the interleukins 6 and 10 in comparison with dogs in other groups.

Inteleukin-6 showed no change in its circulating level 4 h post operativein dexmedetomidine-bupivacaine and ketamine-bupivacaine groups. For fentanyl-bupivacaine and bupivacaine groups IL-6 showed a high level. 24 h post epidural administration of the different protocols, normal level of IL-6 had been recorded in dexmedetomidine-bupivacaine group in contrast with ketamine-bupivacaine, fentanyl-bupivacaine or bupivacaine groups which showed a rise in circulating IL-6.

Inteleukin-10 showed no change in its circulating level 4 h post operativein dexmedetomidine-bupivacaine and ketamine-bupivacaine groups. For fentanyl-bupivacaine and bupivacaine groups IL-6 showed a low level. About 24 h post epidural administration of the different protocols, normal level of IL-10 had been recorded in dexmedetomidine-bupivacaine group in contrast with ketamine-bupivacaine, fentanyl-bupivacaine or bupivacaine groups which showed a decrease in circulating IL-10.

The results of ILs-6 and 10 were similar to those observed by (Kang *et al.*, 2013) in their study thatdexmedetomidine administration during surgery reduced intraoperative and post-operative secretion of cytokines; The pro-inflammatory cytokines tumour necrosis factor -α, interleukin -1β and IL-6 and anti-inflammatory cytokines IL-4 and IL-10, as well as post-operative leukocyte count and CRP level. Garcia *et al.* (2002), in their study showed no preemptive effect of epidural fentanyl plus bupivacaine on postoperative pain and stress response as measured by IL-6 concentrations. Ward *et al.* (2011) proved that lipopolysaccharide increased the serum concentrations of interleukin (IL)-1α, IL-1β, IL-6, IL-10, tumor necrosis factor-α and interferon-γ but Ketamine dose dependently attenuated these changes.

Silva *et al.* (2012) concluded that the epidural injection of 25 mg S(+)-ketamine before incision reduced the pain intensity only 12 h after surgical incision and did not alter concentration of cytokines (IL-6, TNF-α and IL-10). While Carvalho *et al.* (2010) demonastrated that bupivacaine wound infusion resulted in a significant decrease of interleukin 10 and increase of substance P in wounds compared with saline infusion, these findings was similar to our as bupivacaine resulted in decrease level of circulating IL-10 4 and 24 h post operative.

5. CONCLUSION

Finally we recommend the use of epidural bupivacaine-dexmedetomidine combination to provide long term analgesia which continued up to the first 24 hrs post- operative without additional analgesics. Also Further study is needed because the current study was limited by; small sample size as well as further objective evaluations such as acute phase proteins, C-reactive proteins and fibrinogen which should be measured for a longer period until the return of biochemical measurements to normal level so that we can monitor the impact of the surgical castration on dogs.

6. ACKNOWLEDGEMENT

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